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MENINGES AND CEREBROSPINAL FLUID[1]

By LEWIS H. WEED

Department of Anatomy, John Hopkins University

THE divorce of structure from function is particularly difficult in any anatomical study: it was only 85 years ago that the two subjects of morphology and physiology were considered to justify separate departments as academic disciplines. But with this cleavage which fortunately has not at any time been a rigid one, only certain investigations could go forward without loss of inspiration and interpretation when studied apart from the sister science; other researches were enormously hampered and could be attacked only with due regard to structure and function. So it is without apologies that I begin the presentation of the problem of the coverings of the central nervous system —coverings which encompass a characteristic body fluid. Here then is a problem of membranes serving to contain a clear, limpid liquid as a sac might hold it. Immediately many questions of biological significance are at hand: how does it happen that these structures retain fluid; where does the fluid come from; where does it go; is the fluid constantly produced or is it an inert, non-circulating medium; is the fluid under pressure above that of the atmosphere; does it move about with changes in the animal body?—but the list of problems springing into one's mind grows too long.

Knowledge regarding these many questions has progressed since the first accounts of hydrocephalus were given by writers in the Hippocratic corpus, since discovery of the normal ventricular fluid in Galen's time, since its meningeal existence was first uncovered by Valsalva (1911) and advanced by Cotugno (1779), since the first adequate description by Magendie (1825) 100 years ago. We know now that the ventriculo-meningeal system is not like the coelomic serous sacs which we designate as pleural, pericardial and peritoneal cavities, for in these cavities no specialized mechanisms for the production and absorption of the fluid exist. We know now that this fluid-space around the neuraxis differs from the synovial cavities, mucous bursae or tendon sheaths, all of which may possibly be grouped together in a common loose classification. We know now that the fluid-relationships about the nervous system have greatest similarity to those of the aqueous humour of the eye, with an origin from a specialized structure and a process of absorption through a differentiated structure. But let us examine the evidence for these generalizations about the meninges and the contained fluid—have we even now the right to

[1] This survey of the problems and investigations arising out of the cerebrospinal fluid formed the basis of lectures given in the Anatomy Department of University College by Prof. L. H. Weed at the invitation of the London University.

be at all dogmatic about our knowledge of the cerebrospinal fluid and its pathways?

It is customary to speak of the pathways of the cerebrospinal fluid as being of two parts—the ventricular and meningeal. The cerebral ventricles all form part of the first pathway, communicating as they do with each other and including within them the chorioid plexuses. The ventricles are everywhere lined with a typical cuboidal or low columnar cell, the ependymal cell, which in the adult simulates closely its initial embryonic appearance. Over the vascular tufts of the chorioid plexuses of the ventricles, the ependymal cell becomes specifically differentiated into a high columnar type.

The second part of the cerebrospinal pathway is that within the meninges; of these membranes the customary anatomical description lists three. The outermost, the dura mater, is a strong, thick and dense membrane of which the cranial portion is usually depicted as being composed of two layers; these lamina split to enclose the large venous sinuses and certain other structures. In the spinal region these two layers are not in approximation, for the outer layer is taken to constitute the internal periosteum of the vertebral canal while the inner fraction becomes the true spinal dura mater. Between these two so-called layers of dura in the vertebral canal occurs the epidural space, filled in the mammals with fatty areolar tissue and thin-walled veins. Within the cranium the dura adheres closely to the inner surface of the bones forming the skull cavity, serving there as the internal periosteum.

Histologically the dura mater is composed almost exclusively of dense bands of white fibrous tissue (as shown beautifully by Key & Retzius, 1876) with but few elastic fibrils; the dense bands interlace in every direction and give no indication of a cleavage into two portions. The nuclei are sparse in number and are typical of dense fibrous membranes elsewhere in the body. The inner surface of the dura mater is covered by flattened, polygonal mesothelial cells with relatively large oval nuclei. To-day we are forced to believe that no true lymphatic vessels (those possessing an endothelial lining) exist within the dura mater.

The framework of the second, or middle, of the three meninges, the arachnoidea, is a delicate fibrous matrix composed of white fibres with an admixture of elastic elements. This framework forms a continuous sheath for the arachnoid membrane and projects inward as irregular cores for the arachnoid trabeculae. Reaching the surface of the brain, the trabecular cores blend with the subpial network. Upon this reticular base is superimposed the essential cell-element of the leptomeninges. This cell is structurally identical with that lining the inner surface of the dura, being of a flattened mesothelial type and showing characteristic functional changes in morphology (Essick, 1920; Woollard, 1924; Kubie & Schultz, 1925).

These mesothelial cells form a continuous layer over the outer surface of the arachnoidea, covering likewise the inner surface of this membrane and extending inward as a continuous cellular sheath over the arachnoidal trabe-

culae. As the trabeculae merge with the pia mater, these covering cells spread out over this innermost membrane. The pia mater is not, however, a continuous membrane, as is the arachnoid, for the pia is pierced by the perivascular cuffs of the entering and energent blood vessels and probably is perforated in the rhombencephalic roof areas.

Although in general the dura mater and the arachnoidea constitute definitive membranes separated by the fluid-containing subdural space, there are points of fusion between the two structures. In every case it is simplest to look upon these points of fusion as invasions of the dense dura mater by the arachnoidea, though such a description of the process is wholly arbitrary and probably incorrect embryologically.

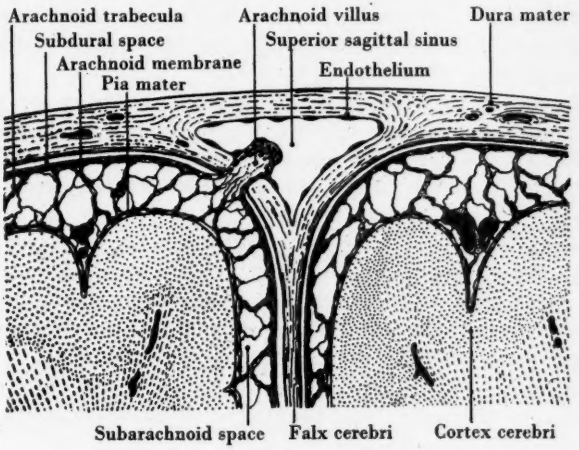

Fig. 1.

The first of these processes of fusion are the structures known as the cranial arachnoid villi—the microscopic precursors of Pacchionian granulations, recently well studied by LeGros Clark (1920), and by Winkelman & Fay (1930). The villi are essentially continuations of the arachnoid mesh into the lateral walls of the great dural sinuses, so that the arachnoid mesothelium comes to lie directly beneath the vascular endothelium. The core of the villus may be a loose strand-like network or a reticular tissue possessing myxomatous characters. The arachnoid cells at the tip of the villi, directly beneath the vascular endothelium, are usually several layers in thickness, thus forming cellular caps over the villi. The microscopic arachnoid villus is found at all ages in man and in all mammals thus far studied, and it probably occurs throughout all orders of mammals. Essentially similar structures have been described by Elman (1923) as projecting from the arachnoid into each segmental vein throughout the spinal region.

The second type of contact between dura and arachnoidea is achieved through the so-called arachnoid cell-columns which represent prolongations of the arachnoid mesothelium into the dura in sites other than those along the dural sinuses. In this structural group, the arachnoid mesothelium is found as a solid core of cuboidal cells with large oval nuclei; this cellular core is surrounded by the fibrous tissue of the dura.

It seems unnecessary to describe at length the blood vessels of the central nervous system and meninges, as only a few characters of these vessels are involved in the questions under discussion. Most important of the anatomical features having to do with the cerebrospinal fluid is the fact that any vessel transversing the subarachnoid space is covered by arachnoid mesothelium. A second feature which is directly related to our main problem is that ultimately the venous drainage of the cranial vascular bed is through the dural sinuses, enclosed within the layers of the dura and having relatively little, if any, power of independent change of calibre.

With this brief description of the anatomical features of the ventriculomeningeal system, our discussion now passes to the problem of formation of the cerebrospinal fluid. Here the task is one of weighing evidence from many sources in biology; almost none of the specific observations is in itself conclusive proof of the site or mode of production of the fluid. Examination of these pertinent data from diverse fields leads inevitably to the view that the elaboration of the greater volume of the fluid takes place within the cerebral ventricles, and that the chorioid plexuses are the responsible structures even though final demonstration of this relationship has been lacking until recently.

The description of the glandular histological structure of the chorioid plexuses by Faivre (1853) marked the discarding of the older concept of Haller (1757) and Magendie (1825) that the cerebrospinal fluid was a product of the leptomeninges (particularly of the pia mater). Faivre made the first histological survey of these villous projections, showing that the cell-coverings were epithelial in nature and that they contained inclusions which could be interpreted as indicating secretory activity in the cells. Faivre's observations, supported by similar microscopic studies by Luschka (1855), gave origin to the hypothesis that the chorioid plexuses of the cerebral ventricles elaborate the cerebrospinal fluid; this has remained the hypothesis upon which most of the investigations regarding the source of this peculiar body-fluid have been based.

The purely histological evidence presented in support of this hypothesis, while suggestive, lacks many elements of proof, though for many years accepted without question. During the past 30 years, renewed attempts (*vide* Weed, 1922; Flexner, 1934) with histological and cytological methods have been made to bridge the gap between intracellular secretion-granules (vacuoles) and the actual production of a fluid surrounding the cells. Thus there have been many descriptions of pigmented globules, of hyaline-like bodies, of osmic-staining granules, of lipoid inclusions, of glycogen plaques or particles, of

Golgi apparatus, of fuchsinophilic granules, of basophilic plasmosomes, of nuclear granules, of mitochondria, etc. In all of these studies, there is no irrefutable evidence that these intracellular structures constitute the intracellular mechanisms for the elaboration of the cerebrospinal fluid. The difficulty of final demonstration that these granules are absorbed into the fluid, or discharged into the fluid, cannot be surmounted with present-day histological methods.

Fortunately observations of a somewhat more conclusive nature are available through combination of histological and pharmacological methods. In this category are the descriptions of Pettit & Girard (1902), who reported an increase in volume of the chorioidal cytoplasm after injections of muscarin, pilocarpine, etc., each of which Cappelletti (1900) supposed to increase the production of cerebrospinal fluid. As these drugs are largely those which stimulate other ducted glands to secrete, Pettit & Girard's findings (as confirmed by Meek (1907)) were considered to constitute proof that a secretory function was lodged in the chorioid plexus. Changes in these epithelial cells, in every way similar to those reported, were found by me, in a later study (1923 *b*), to result from the intravenous injection of large quantities of a hypotonic solution.

Certain indirect support of the relationship between the chorioid plexuses and the cerebrospinal fluid was obtained some 20 years ago in my investigation (1917) of the embryology of the meningeal spaces. It was shown that up to a stage of 14 mm. in pig embryos the growth of the cerebral ventricles kept pace with the production of the fluid within them. In slightly larger embryos a sudden expulsion of the fluid from the ventricles into the developing subarachnoid space occurred. At this stage of growth the chorioid plexuses are just beginning to become tufted; the balance between ventricular growth and production of fluid is abruptly disturbed even though the chorioidal cells are at this stage incapable of producing a cerebrospinal fluid with the physical characters of the adult fluid.

But the chorioid plexuses become more intimately related to the production of cerebrospinal fluid when one considers data from the general field of pathology. Ever since the earliest observations upon hydrocephalus, it has been realized that the cerebrospinal fluid must, at least in part, be produced by some intraventricular structure. Attention was focused upon the chorioid plexuses by the discovery (Claisse & Levy, 1897) of a case of internal hydrocephalus associated with hypertrophy of these intraventricular structures. The experimental production of an internal hydrocephalus by Dandy & Blackfan (1914, 1917), and by Frazier & Peet (1914), gave additional support to the general contention of intraventricular production. Cushing's (1914) observation of an exudation of a clear fluid from the chorioid plexus, exposed in exploration of a porencephalic defect, likewise added suggestive substantiation of the hypothesis, though the operative exposure of the plexus unquestionably altered pressure conditions. Dandy's (1919) later experiment

consisted of the production of a unilateral internal hydrocephalus by obstruction of one foramen of Monro; when this plugging of the foramen was accompanied by extirpation of the chorioid plexus, no ventricular dilatation followed. These observations by Dandy cannot be looked upon as demonstrating the production of the fluid by the plexuses. The lack of histological control in his experimental animals where the chorioid plexuses were supposedly extirpated and the possibility of an intracortical escape of fluid through the operative wound vitiated many of the conclusions.

Somewhat more tangible evidence of intraventricular production of fluid may be obtained from those cases of internal hydrocephalus where an obstruction has been produced in the subarachnoid space. By subarachnoid injection of lamp black a rapidly forming internal hydrocephalus in young animals may be brought about (Weed, 1919). In these cases the chorioid plexuses apparently continue to elaborate fluid at a normal rate or at a rate which becomes slowed as the internal pressure of the ventricles increases. Histological study shows the plexuses to be quite normal even though the intraventricular pressure is approximately half again as high as in the normal controls of the same litter (Nanagas, 1921).

From the field of experimental physiology have come other data pertinent to our discussion. While working in Harvey Cushing's laboratory I was able to record (1915) the outflow of cerebrospinal fluid from a catheter inserted into the third ventricle through the aqueduct of Sylvius and completely closing the aqueduct. Variations in rate of outflow were recorded over a period of hours, demonstrating an intraventricular source of the fluid. More recently, Flexner & Winters (1932) in the anatomical laboratory in Baltimore greatly improved the procedure and were able to measure the intraventricular production of cerebrospinal fluid under standard physiological conditions.

Flexner (1934) has developed an approach to this problem through the chemistry of the cerebrospinal fluid and is even now engaged on a study of the distribution ratio, between blood plasma and cerebrospinal fluid, of chloride, sodium and urea. Using pig embryos as sources of his blood and cerebrospinal fluid, Flexner has found that in stages up to 50 mm. the distribution of diffusible ions on the two sides of the chorioid membrane is almost exactly in agreement with the prediction of the Donnan membrane-equilibrium. At stages between 50 and 60 mm. growth, the chloride ratio of approximately 1 changes suddenly to 1·3, indicating that a new process of production of fluid has been accomplished by some intraventricular structure. At the same time in embryonic growth, the sodium and urea ratios become characteristic of adult animals. Flexner has furthermore discovered that at this critical stage of growth the indophenol oxidase reaction abruptly becomes positive in the cells of the chorioid plexus; quite similarly tissue oxygen uptake, as determined by the Barcroft-Warburg apparatus, shows augmentation. Flexner's histological studies, still incomplete, have thus far yielded no evidence of cellular change in the chorioid plexuses which would account for this shift from a

process of ultrafiltration between the two sides of the membrane to a process of secretion.

Table I. *Chemical analyses. Fluids of pig embryos*
(L. B. Flexner)

C.R.	Distribution between C.S.F. and plasma of		
cm.	Cl	Urea	Na
3·5	1·02	1·02	—
3·7	1·00	1·00	1·02
3·9	1·00	1·00	0·99
4·7	1·01	0·99	1·00
5·0	1·01	0·97	1·00
6·0	—	0·78	1·10
6·5	1·19	0·86	1·15
7·0	1·12	—	1·10
10·0	1·20	0·63	1·09
12·0	1·30	0·78	1·12
20·0	1·25	0·80	1·16

Taking the data as a whole, whether from histological, pharmacological, pathological, or embryological standpoints, one is surely inclined to accept the intraventricular source of the fluid as established and to consider as the most likely hypothesis the production of fluid by the cells of the chorioid plexuses. Flexner's recent observations, still under way in my laboratory, seem to me to relate the plexuses more definitely to the production of fluid than has any other single study. The shift in chloride ratio between blood and cerebrospinal fluid occurs simultaneously with the appearance of the oxidase reaction in the chorioidal epithelium—a correlation which can hardly be explained by chance. With the exception of these new findings by Flexner, it would not seem justifiable to accept the data from any one of the standpoints as conclusive for many of the observations are corroborative only; but the weight of evidence supporting the view of chorioidal production of fluid is far in excess of that in favour of other hypothesis. Yet some workers (Hassin, 1933) in the field remain unconvinced, as may be witnessed by the interpretation of inclusions within the cells of the chorioid plexuses (particularly blood pigments) as indicating that these vascular tufts with their cylindrical epithelium are organs for the absorption rather than the production of fluid. These data seem to me to be without significance as these findings may well be looked upon as the result of phagocytosis of foreign materials in the ventricular fluid.

Accepting momentarily the hypothesis that the major portion of the cerebrospinal fluid is elaborated by an intraventricular structure, the problem of the mechanism of production of this clear liquid immediately presents itself. Since the earliest histological descriptions, repeated controversies have raged as to whether the fluid is a secretion or a filtrate. Until recently no data of permanent significance were advanced, but during the last few years many chemists and physiologists have attempted to reach a conclusion in this important question. The essence of this problem is the determination of the rôle played by the membranes separating blood and cerebrospinal fluid in the

distribution of ionic and molecular species between the two fluids. The problem particularly concerns itself with the question as to whether the cells of the plexus, the walls of its capillaries, and the connective tissue of the stroma of the plexus perform work in the production of cerebrospinal fluid, or whether substances are distributed between blood and cerebrospinal fluids as would occur across an inert lifeless membrane. A large group of investigators, particularly Fremont-Smith and his associates (1925 *a*, *b*; 1931 *a*, *b*), has concluded that the distribution of all diffusible ions between the two fluids is in agreement with the predictions of Donnan's law; cerebrospinal fluid by their interpretation becomes a dialysate of the blood. But this general conclusion has been contradicted by an analysis of the chemical data of the two fluids by Flexner (1934), working in my laboratory. Flexner has treated these data from a thermodynamic standpoint: he has been able to show that approximately 13 calories of energy are required for the formation of a litre of cerebrospinal fluid. A free energy change of only 0·9 of a calorie could be explained by capillary pressure. Ultrafiltration (i.e. diffusion plus hydrostatic pressure) Flexner therefore judged to be an inadequate explanation of the elaboration of the fluid. On the basis of present evidence, Flexner concluded that the cerebrospinal fluid is to be considered a secretion, using that term to mean that the cells do work in its production. Flexner's analysis of the problem is of utmost significance in the present stage of our knowledge of the cerebrospinal fluid.

If we may now accept as a fairly well-founded hypothesis the secretion of cerebrospinal fluid by the chorioid plexuses, these epithelial structures must not be considered to be the sole elaborators of the fluid even though the quantity produced by them is far in excess of that from any other source. Over 20 years ago I presented evidence (1914 *b*) that the perivascular spaces of the central nervous system also pour a small amount of fluid into the subarachnoid space. This conclusion was based largely upon subarachnoid injections of true solutions of foreign salts which could be precipitated in situ for subsequent histological study. None of this foreign solution was observed within the perivascular and perineuronal spaces under normal pressure-relationships; but when these pressure-relationships were altered by cerebral anaemia or by dehydration of the nervous system, the foreign solution was aspirated from the subarachnoid space into the perivascular cuffs, even into the spaces around the nerve cells. The evidence that this minimal amount of fluid passes from nerve cell to the subarachnoid space has been greatly strengthened by observations of Kubie (1928), and Kubie & Retan (1933), who have been able to wash out the cell-content of these perivascular spaces by repeated lumbar punctures or by intravenous injection of hypotonic solutions. This perivascular fluid seems to represent an addition to the ventricular cerebrospinal fluid and probably accounts for the many reported differences between subarachnoid and ventricular fluids on serological and chemical analysis. Also in any discussion of the source of the cerebrospinal

fluid some note must be taken of the potentiality of the ependymal cells lining the cerebral ventricles and the central canal of the spinal cord, for these ectodermal cells may also contribute, even in the adult, a minimal addition to the intraventricular cerebrospinal fluid.

The cerebrospinal fluid is passed by the cells of the chorioid plexuses, which do work in the process (Flexner, 1934), in small amount into the neural cavities. According to observations made by Flexner & Winters (1932) in the anatomical laboratory in Baltimore, 12 c.c. of fluid are produced each day in a cat. As approximately one-tenth of the intradural volume of a mammal is represented by the cerebrospinal fluid, the secretion of 12 c.c. each day in a cat with an intradural volume of 35–40 c.c. means a total replacement approximately every 6 hours. If we take as an average volume of cerebrospinal

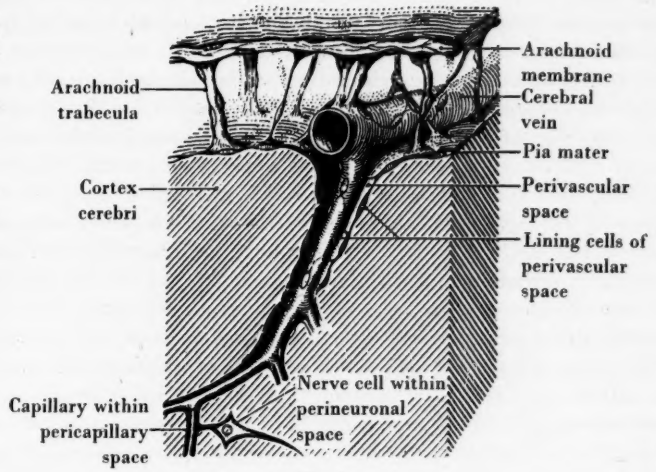

Fig. 2.

fluid in man the figure of 135 c.c., the formation of fluid each day in man would be between 525 and 550 c.c. This is not a rapid process but it is a process which is fairly constant over hours, though, as Flexner & Winters showed, there are periods of a few minutes' duration when no fluid is formed.

That portion of the fluid elaborated in the lateral ventricles flows through the foramina of Monro into the third ventricle and thence by the aqueduct of Sylvius into the fourth ventricle. From the fourth ventricle, the fluid passes out into the subarachnoid space; there is no evidence that functional communications between the cerebral ventricles and the subarachnoid space exist elsewhere than in this region. The exact mode of escape of the ventricular cerebrospinal fluid from the fourth ventricle into the subarachnoid space must still be considered as uncertain. It is possible that the inferior velum of the cerebellar roof in the adult is an intact though functioning membrane as

in the embryo. When studying the embryology of the subarachnoid space (1917), I ascertained that at the stage of approximately 14 mm. in the developing pig, aggregations of a precipitate from a foreign solution introduced in the cerebral ventricles and in the spinal canal system appeared in the ventricular roof; it was found that these aggregations of precipitate occurred in two areas which were differentiated histologically from the lining ependyma. At a little later stage, embryonic ventricular fluid passed through these definitive areas, as through an intact though permeable membrane; at still larger stage (approximately 26 mm.) when the subarachnoid space was completely outlined by the foreign solution, the membrane of the ventricular roof was still intact and permeable to proteins and to foreign salts. In embryos up to and including 50 mm. in the pig and man, the ventricular roof was found to be a bulging, thin membrane composed of a layer of ventricular ependymal cells and pial mesothelial cells. Whether this thin membrane breaks down to form a foramen of Magendie, as a true anatomical opening in the velum, is, in my opinion, to-day not conclusively proved, as the technical procedures available are not adequate for the purpose of demonstration. The two foramina of Luschka, connecting the lateral recesses of the fourth ventricle with the subarachnoid space, seem to have as established a basis for their existence as does the medial foramen. It is through these three foramina—or surely in the region of the inferior tela chorioidea if through an intact membrane—that the cerebrospinal fluid produced in the cerebral ventricles passes into the subarachnoid space.

From the cisternal dilatation of the subarachnoid space in the region of the medial cerebello-bulbar angle the cerebrospinal fluid very slowly seeps downward in the spinal subarachnoid space but passes more rapidly up about the base of the brain and thence more slowly over the hemispheres, thus surrounding the whole central nervous system. This movement of fluid is in large measure activated by a *vis a tergo* from the point of production in the cerebral ventricles. In the spinal region there may be an equivalent passage of fluid upward. The cerebrospinal fluid then circulates everywhere about the central nervous system both in the cerebral ventricles and in the tortuous meshes of the subarachnoid space. These channels are all clothed with a specialized cell, fluid-retaining so that a true circulation of fluid may be maintained; and in these channels the fluid finally comes into close relationship to the venous system through the arachnoid villi.

It is now 25 years since I embarked upon an investigation of the meninges and cerebrospinal fluid. The first problem which I undertook was that of determination of the method of return of the cerebrospinal fluid to the venous system. At that time there were several views of the anatomical pathways concerned in this progress. In the 1870's, Key & Retzius (1876) had introduced gelatine solutions, coloured with blue, into the spinal subarachnoid space of a cadaver and had been able to trace the gelatine throughout the whole subarachnoid space and finally through the Pacchionian granulations into the great dural sinuses. Unfortunately, Key & Retzius used high pressures of injection

(about 60 mm. Hg) on non-living subjects and their drawings of the Pacchionian granulations show evidence of rupture of the structures.

Key & Retzius' view that the cerebrospinal fluid was returned to the blood stream through these arachnoid structures was accepted for many years, but the failure to discover gross Pacchionian granulations in the human infant and in the four-footed mammals had led to gradual abandonment of the hypothesis. Cushing (1902), following the rupture of a mercury balloon within the meninges, had hypothesized the occurrence of a valve-like mechanism between the subarachnoid space and the cerebral sinuses; while Mott (1910), on the basis of histological studies of brains from animals subjected to experimental cerebral anaemia, had assumed that the cerebrospinal fluid returned to the blood stream by way of the perivascular spaces into the cerebral capillaries.

At that period, there were many physiological experiments indicating a very rapid passage of foreign solutions from the subarachnoid space into the venous system. Thus Reiner & Schnitzler (1894) detected potassium ferrocyanide in the jugular blood stream 10 sec. after subarachnoid injection. Hill (1896) was able to trace methylene blue, as he described it, "straight into the venous sinuses" from the cerebrospinal spaces. Ziegler (1896) and later Lewandowsky (1900) made similar observations of rapid passage of foreign true solutions into the cerebral veins after subarachnoid injection. At the same time, the evidence had become fairly strong that suspended material (carbon particles, cinnabar granules, etc.) could not pass into the venous system. Observations made with vital dyes, such as trypan blue, had not demonstrated an anatomical pathway, both because of the intraspinous toxicity of the substances and because of the affinity of certain lining cells for the dye. There were however accumulating certain data which indicated that, in addition to the major absorption of the cerebrospinal fluid into the venous system, there existed a minor pathway of absorption into the lymphatic system.

It was upon this background that I started to work upon the anatomical pathways for absorption of the cerebrospinal fluid (1914 *a*, *b*, *c*). It was clearly evident that morphological methods alone would not give reliable data; these methods of study had to be combined with a physiological approach to the whole problem. It was felt that a solution of foreign electrolytes which could be precipitated in situ for subsequent histological examination, offered the best chance of successful demonstration of the channels of fluid-passage. In addition, it was held that any injection into the subarachnoid space must be made at a pressure approximating that of the cerebrospinal fluid (i.e. in the neighbourhood of 125 mm. saline). As a control, certain replacement-experiments, where the spinal subarachnoid fluid was withdrawn and replaced by a foreign identifiable solution, were projected. Attempts were made with various foreign salts but almost all were discarded because of toxicity, or because of inability to form insoluble precipitates which could be subjected to the various technical procedures and still be identified in histological sections. Finally potassium ferrocyanide and iron-ammonium citrate in isotonic solution were

found to be satisfactory. These foreign salts were introduced into the subarachnoid space of anesthetized mammals over periods of several hours at pressures approximating the normal. At the end of the time of injection, the animals were killed and the head of the animal quickly severed from the body and placed in an acidulated formalin solution; or in the second series, the acidulated formalin was injected into a carotid artery immediately after death. In these two ways fixation of the tissues was fairly prompt and the ferrocyanide solution was precipitated in situ as insoluble Prussian blue.

Subsequent study of the microscopic sections of the central nervous system and meninges showed that the ferrocyanide precipitate could be identified throughout the subarachnoid space. Everywhere in this space the Prussian blue granules were seen adhering to the inner surfaces of the lining mesothelial cells. In no case where fixation and precipitation were prompt was there evidence of penetration of the cell-membranes of the lining cells by the foreign solution. Most important was the finding of the precipitate in large amount in the cranial arachnoid villi, those projections of arachnoid directly beneath the endothelial walls of the dural venous channels. The foreign solution as represented by finely dispersed granules could be followed through the mesothelial cells at the cap of the villus as well as through the single-layered endothelial lining of the vessel.

No other pathway of direct absorption into the cranial blood stream was found anywhere in the material examined. No structure which could serve as a valve-like mechanism between subarachnoid space and venous sinuses was discovered. And in no case where normal pressure-relations between subarachnoid space and venous system were maintained was there evidence of passage of the foreign solution from the subarachnoid space inward along the perivascular spaces to the cerebral capillaries.

In addition to the passage through the villi into the great venous sinuses from the cranial subarachnoid space, there was indication of a slow accessory absorption of the fluid into the lymphatic system of the body. This secondary pathway seemed to be through perineural spaces for a limited distance outward along the spinal and cranial nerves, and then an indirect passage through tissue-spaces into the adjacent lymphatic vessels. This process was especially evident around the olfactory fila in the nasal mucous membrane. It appeared, however, to be in every way an indirect, accessory mechanism of absorption for the cerebrospinal fluid.

These observations, when considered with other evidence, indicated that the cranial end of the central nervous system provided by far the greatest area of absorption. Dandy & Blackfan (1914, 1917) had previously reported an absorption of phenolsulphonphthalein from the isolated spinal subarachnoid space quantitatively as great as from both cranial and spinal spaces. Their interpretations were apparently based on the erroneous supposition that replacements of 1 c.c. of spinal subarachnoid fluid with the foreign solution could be made without increase of subarachnoid pressure or leakage

into the epidural tissues about the puncture-wound. In my hands (1914 *b*) reversal of the experiments (i.e. injection of the same dye-stuff cephalad to the spinal ligature) showed that the cranial end of the nervous system afforded an absorptive bed so large that the exclusion of the isolated spinal subarachnoid space did not affect the quantity of the dye recovered.

While these data strongly suggested the cranium as the site of absorption of most of the cerebrospinal fluid, there was still the possibility of a pathway within the spinal subarachnoid space. This problem was attacked in my laboratory by Elman (1923), who, as mentioned previously, discovered that there were spinal arachnoid villi which projected into each of the segmental veins from the arachnoid cul-de-sac. By similar methods of ferrocyanide injections in living animals, Elman demonstrated that absorption takes place in the spinal region through these structures, just as the passage of the foreign salts into the great dural sinuses occurred. The spinal villi are however minute and the percentage of total absorption of cerebrospinal fluid going on by way of these villi can only be small.

Since the publication of these findings in 1914, there has been fairly general acceptance of the observations. In 1923 (1923 *a*) I restudied the question, relying on replacement-injections of the ferrocyanide solution and recording arterial and venous pressures. The findings under these conditions were essentially the same as in the experiments of 10 years previous. But the general idea of passage of cerebrospinal fluid through those specialized structures, the arachnoid villi, was questioned by Dandy (1929). His experiments consisted in freeing the brain from its connexion with the sagittal, circular and transverse sinuses, leaving the pia-arachnoid seemingly intact. As no discomfort in the experimental dogs was subsequently observed, Dandy was led to conclude that the arachnoid villi played no rôle in absorption. Such a conclusion seems unwarranted as it takes no account of the fact that the animals possessed intact spinal villi; it takes no account of the possibility of regeneration or re-establishment of channels from the arachnoid membrane into the dural sinuses; it takes no account of the fact that an increased pressure of the cerebrospinal fluid may lead to the same rate of absorption with smaller absorptive surfaces. No histological findings were published by Dandy so that it is impossible to ascertain the physiological or reparative processes within the crania of the experimental animals.

At the moment we cannot regard as established any theory of drainage of cerebrospinal fluid other than the suggested one of absorption through arachnoid villi directly into the venous system. We must, however, recognize fully the limitations of any method of demonstration of a fluid-pathway which depends upon the introduction of foreign salts. I have held it to be particularly desirable (1935 *a*) that some new method of investigation of this problem be devised, as both ante-mortem and post-mortem diffusion of the foreign crystalloids has always to be considered. The difficulty of post-mortem diffusion of the crystalloids now bids well to be eliminated due to the work of

two medical students in my laboratory. Under Flexner's direction, these men, Mr Ralston and Mr Scholtz, have employed a modification of the Altmann-Gersh technique of instantaneous freezing of the tissues in liquid air. Subarachnoid injections, under low pressures and for several hours, of an isotonic solution of sodium ferrocyanide are first made in the anaesthetized animal. While the animal is still alive, the sagittal sinus region of the head is frozen as a block of liquid air, thus preventing diffusion of the foreign salts. The block of tissue, while still frozen, is cut from the animal and dehydrated in a high vacuum at low temperature, $-30°$ C. The dehydrated tissue is embedded in paraffin without use of alcohol or water, and is then sectioned. The sections are treated with ferric chloride which precipitates any ferrocyanide present as an insoluble ferric ferrocyanide.

The use of this technique has given additional evidence in support of the hypothesis that the major pathway of absorption of cerebrospinal fluid is through the cranial arachnoid villi directly into the sinuses, though some diffuse staining with blue occurs about certain of the subarachnoid veins. Mr Ralston's and Mr Scholtz' work is still in progress, and while interpretation of the findings will necessarily depend upon additional data, it seems to eliminate one of the chief objections—post-mortem diffusion—lodged against experiments in which the foreign salts are precipitated after immersion or by arterial injection. There still remains, however, the great desirability of devising some new method of attack upon this problem—a new method which will use other foreign salts with different rates of diffusion, yet capable of precipitation in situ for subsequent microscopic study.

These attempts to determine the pathway of escape of the cerebrospinal fluid into the venous system do not of course take any account of the mechanism of absorption of the cerebrospinal fluid. In my original study (1914*a*, *b*), the underlying physical forces were hardly touched upon, largely because of lack of method of attack. The problem could not be successfully undertaken until means were at hand to measure the absorption of small quantities of fluid from the subarachnoid space under constant pressure-relationships. Fortunately Mortensen and I (1934) were able to devise a simple apparatus which permits the desired measurements

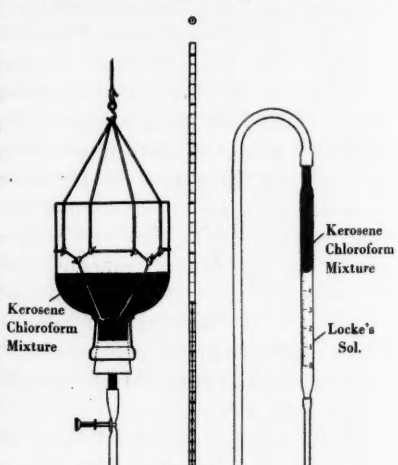

Fig. 3.

under given conditions of pressure. The system (see fig. 3) consists of a pipette, filled with fluid and connected by a three-way stopcock both to the subarachnoid space of the living animal and to a small bore, open-end manometer recording pressure. On top of the fluid in the burette is superimposed a coloured oil mixture of the same specific gravity as Locke's solution. This oil is connected through rubber tubing to a reservoir of such large volume and surface that the absorption of 10–50 c.c. of saline solution from the burette does not appreciably lower the level of the oil mixture.

The employment of this reservoir-pipette system led indirectly to an analysis of the forces concerned in the absorption of the cerebrospinal fluid (Weed, 1935 *b*). The early theories considered that the absorption of cerebrospinal fluid was largely a process of filtration through membranes from a point of higher to a point of lower hydrostatic pressure. These hypotheses were discarded when observations indicated an identity of pressures in torcular herophili and subarachnoid space, but the finding that the venous pressure in the superior sagittal sinus is usually lower than the pressure of the cerebrospinal fluid seemed to lend support to the general contention. Likewise, the many reports that the higher the pressure of introduction the more rapidly is an isotonic foreign solution absorbed from the subarachnoid space suggested an influence of hydrostatic pressures (Mortensen & Weed, 1934).

In considering the factors which might play a rôle in the process of absorption of cerebrospinal fluid it seemed justifiable to assume that the colloid osmotic pressure of the blood and the hydrostatic pressure-difference between the subarachnoid pressure and the intracranial venous pressure should be the effective forces. The crystalloids of the blood and of the fluid are approximately the same in nature and amount, so that no effective pressures could be created by these substances. It therefore appeared feasible to attempt determination of the total effective pressures bringing about absorption of the fluid, for the osmotic pressure of the blood could be ascertained through use of appropriate celloidin membranes and the hydrostatic factor could be had by measurement of the subarachnoid pressure and of the sagittal venous pressure.

The technical procedure on the etherized dog consisted essentially in the connexion of the pipette-reservoir system to the subarachnoid space and the recording of the sagittal venous pressure by appropriate means (Weed & Hughson, 1921 *c*). The normal level of subarachnoid pressure of the animal was first determined and then the pressure in the subarachnoid space was raised by increments of 50 mm. until a maximum pressure of about 600 mm. of saline was attained. At each of these 50 mm. increments in subarachnoid pressure the actual absorption of Locke's solution was determined, together with the corresponding sagittal pressure. As soon as this series of observations had been made, the actual rate of absorption in the same series of established pressures was measured for a protein-solution (gelatine, pure casein, or serum). At the end of the experiment, the colloid osmotic pressure of the blood serum

and of the protein-mixture in the subarachnoid space was determined by celloidin membranes.

From these data, it became possible to calculate the total effective pressures by adding the effective colloid osmotic value (i.e. colloid osmotic pressure of the blood minus that of the subarachnoid fluid) to the effective hydrostatic

Table II

C.S.F. pressure	Sagittal venous pressure	C.S.F. minus sagittal pressure	Colloid osmotic pressure blood serum	Colloid osmotic pressure modified C.S.F.	Colloid osmotic pressure blood minus solution	Total effective pressure	Absorption rate per min.
			Locke's solution				
mm. saline	mm. saline	mm. saline	mm. saline	mm. saline	mm. saline	mm. saline	c.c.
250	149	101	312	—	312	413	0·02
300	154	146	312	—	312	458	0·06
350	154	196	312	—	312	508	0·10
400	148	252	312	—	312	564	0·14
450	146	304	312	—	312	616	0·19
500	145	355	312	—	312	667	0·23
550	155	395	312	—	312	707	0·27
600	180	420	312	—	312	732	0·28
			Gelatin solution				
300	137	163	312	98	214	377	0·003
350	150	200	312	98	214	414	0·03
400	144	256	312	98	214	470	0·07
450	135	315	312	98	214	529	0·11
500	145	355	312	98	214	569	0·15
550	152	398	312	98	214	612	0·18
600	149	451	312	98	214	665	0·23

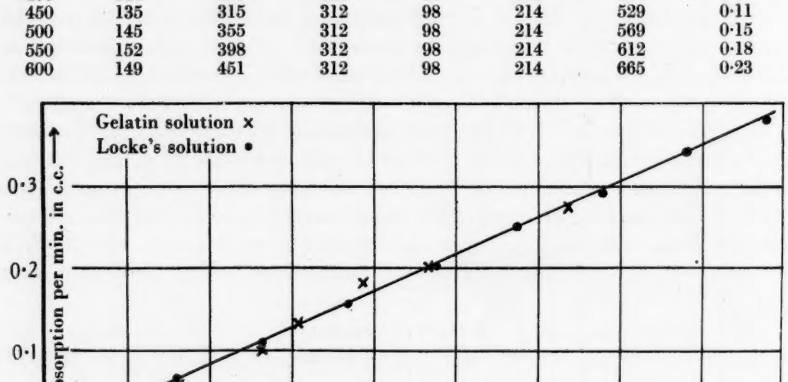

Fig. 4.

pressure which was derived by substracting the sagittal venous pressure from the established subarachnoid pressure. When the comparative absorption rates of the Locke's solution and of the foreign protein-solution were plotted against the total effective pressures, a linear relationship for both the Locke's and the protein-solutions was apparent.

These findings, that the rates of subarachnoid absorption of an isotonic crystalloid solution and a protein-containing solution when plotted against total effective pressures are described by the same straight line, would indicate that the factors assumed to make up the "total effective pressure" are actually the ones responsible for the process of absorption of the cerebrospinal fluid. In spite of all the difficulties and shortcomings of the methods for the determination of colloid osmotic pressures the data when accepted as an average seem adequate. While certain observers have found that during anaesthesia the colloid osmotic pressure of the blood changes, control observations under the same conditions as these experiments have given no indication that this osmotic pressure changes to any appreciable extent during the period of etherization. The data were clear-cut in demonstrating that the rate of absorption of a protein-solution from the subarachnoid space is slower than that of an isotonic crystalloid solution. The effective colloid osmotic pressure of the blood is diminished by the amount of colloid osmotic pressure of the mixture of cerebrospinal fluid and protein-solution: the passage of the fluid (water plus crystalloids) through the absorbing membranes is therefore retarded. It would seem reasonable to assume that protein does not leave the subarachnoid space in appreciable quantity during the short period of introduction of the foreign solution. The regularity of the experimental data indicates that there is no material change of protein concentration in the subarachnoid space during the period of measurement. This constancy seems to be a function of the total quantity of the protein-solution in that space (relatively large at high pressures apparently) and of the amount of water abstracted from the protein-solution by absorption. The experiments were apparently executed rapidly enough for the amount of absorption of water to make no detectable difference in the protein concentration.

Further experiments with hypertonic and hypotonic solutions of crystalloids have indicated a rate of subarachnoid absorption exactly that of the isotonic solution. A linear relationship between absorption rate and effective pressure was apparent. The explanation for these extraordinary results with distilled water and Locke's solution of double salt concentration is not clear: it may be that such solutions within the subarachnoid space are quickly rendered isotonic. Recently further extensions of these experiments have been made using sucrose solutions for comparison with the isotonic solutions in their absorption rate. With 5 % solution of this sugar in Locke's solution, the rate of absorption from the subarachnoid space was found to be much slower than was the case with the normal Locke's solution. When plotted against total effective pressure its rate of absorption formed a straight-line relationship but at a far lower level than with the Locke's solution. Here the large molecule of sucrose apparently exercised an osmotic pull against the colloid osmotic pressure of the blood, decreasing the effective pressure over the short period of the experiment (30 min.).

From an analysis of the data in this series of experiments it seems fair to

conclude that the total effective force actuating the normal process of absorption of the cerebrospinal fluid is compounded of the colloid osmotic pressure of the blood plus a hydrostatic factor derived from the difference in subarachnoid pressure and intracranial venous pressure.

Hitherto it has been necessary to refer to pressure conditions within the cranium, and possibly some confusion has arisen because of reference to the pressure of the cerebrospinal fluid and of the intracranial venous system without more detailed discussion. The idea of equality of pressures between the cerebrospinal fluid and the cerebral veins as advanced by Hill (1896) was abandoned following upon the work of Dixon & Halliburton (1914).

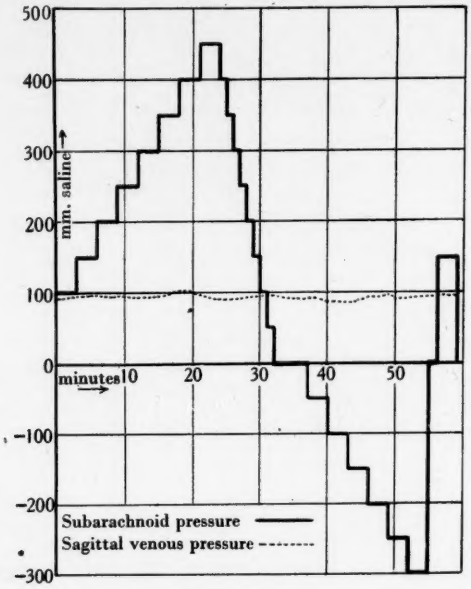

Fig. 5.

Since that time current conceptions have acknowledged an independence of the two pressures. In the horizontal position, four-footed mammals (especially the common experimental animals, cat and dog) exhibit a pressure of the cerebrospinal fluid which is customarily slightly higher than that in the superior sagittal sinus. An average value of the cerebrospinal fluid pressure in the cat and dog is 125 mm. of saline, while the sagittal pressure usually ranges 15–50 mm. below this level.

The fluid's pressure is momentarily affected by rapid changes in the cerebral arterial pressure but not by slowly effected alterations. Changes in the pressure of the cerebral veins are generally held to alter the pressure of the cerebrospinal fluid, always in the same direction and to a lesser extent. Conversely,

alteration of the pressure of the cerebrospinal fluid has been assumed by many workers to be reflected in the pressure of the cerebral veins, but conflicting reports of this possible effect are found in the literature. These generalizations imply that a condition of pressure-equilibrium exists between the two fluids (cerebral venous blood and cerebrospinal fluid) which are separated by a membrane comprised of vascular and meningeal tissues and that this equilibrium can be shifted by change in either of these pressures. Such alteration of either pressure would in this interpretation lead to a new equilibrium, characterized by change in the same direction of the other pressure and by a shift in position of the membrane separating the two fluids.

In an investigation of this general pressure-relationship carried out by Weed & Flexner (1933), it was quickly found that even profound alterations of the subarachnoid pressure have no effect upon the pressure in the cerebral veins. This observation was a confirmation and extension of the results obtained by Becht (1920). This lack of effect of subarachnoid pressure upon the cerebral venous pressure held for all animals in the series during the first 2 hours of etherization; but after this period of prolonged anaesthesia occasional animals showed a slight change in intracranial venous pressure from alteration in subarachnoid pressure. Such reactions were of small extent—10–15 mm. venous pressure-change on subarachnoid pressure-change of 150 mm.—and were interpreted as being due to general fatigue of the animal, particularly of its vasomotor system.

The converse of this physiological phenomenon was found by Flexner and myself to be of a positive nature. Increase in cerebral venous pressure, as effected by obstruction upon the veins of the neck of the animal, results in a quick rise of the pressure of the cerebrospinal fluid, but always to a lesser extent. This

Table III. *Positional pressure-changes in cerebrospinal fluid and superior sagittal sinus, together with volume-changes of the fluid*

		Head down			Tail down		
Condition of animal	Manometer bore mm.	Pressure-change C.S.F. mm.	Pressure change sagittal mm.	Volume dislocated C.S.F. c.c.	Pressure-change C.S.F. mm.	Pressure-change sagittal mm.	Volume dislocated C.S.F. c.c.
Living dog, E 99	1	185	237	0·321	126	204	0·219
	4	102	230	1·163	64	204	0·736
	6	62	229	1·779	33	204	0·947
	8+	33·5	234	2·258	15·5	201	1·045
	10−	30·5	233	2·173	14·5	200	1·033
	12	20·5	235	2·337	9·0	199	1·026
	1*	185	235	0·321	110	198	0·191
Dog, immediately after death, E 68	1	243	325	0·422	234	299	0·406
	4	208	328	2·392	141	304	1·622
	6	145	327	4·162	79	298	2·267
	8+	73	329	4·920	32	295	2·167
	10−	68	325	4·845	33	296	2·350
	12	43	328	4·902	20	297	2·280

* 1 mm. manometer replaced after other manometers were used in series.

reaction is well known experimentally and is employed clinically in the Queckenstedt test. Eleven experiments in our series yielded an average increase of 276 mm. in cerebral venous pressure and 176 mm. in the cerebrospinal fluid pressure, indicating that 65 % of the venous pressure-increase is reflected in the pressure of the fluid. Flexner and I extended our studies to tilting experiments to ascertain the influence of different pressure-changes in the cerebrospinal fluid upon the pressure-changes in the superior sagittal sinus. Data were obtained which indicated that, as in the horizontal position, pressure-changes in the cerebrospinal fluid have no effect upon pressure-changes in the sagittal sinus. In addition, by means of the use of different sized manometers a very definite relationship of pressure-change to volume-change in the cerebrospinal fluid was obtained. At a given point in the individual animal a maximum volume-dislocation of the cerebrospinal fluid is obtained; the employment of larger manometers, permitting outflow of greater amounts of fluid under lower pressure-conditions did not result in displacement of larger quantities of fluid from the animal.

These findings permit an interpretation of the kind of pressure-equilibrium which exists between the cerebrospinal fluid and the cerebral veins. Any intracranial venous pressure-change results in change of the cerebrospinal fluid pressure, about six-tenths of the venous pressure-change being effective in the fluid when the fluid-dislocation is minimal. With the experimental increase of cerebrospinal fluid pressure brought about by the introduction of fluid into the subarachnoid space, a certain diminution of intradural venous volume must follow; but the pressure in these cerebral veins remains unaltered. Conversely, when the pressure of the cerebrospinal fluid is reduced mechanically, or by tilting, the cerebral venous bed reciprocally compensates by dilatation, the veins expanding and permitting an increase of intradural venous volume though maintaining in these expanded channels the same pressure. Under both these conditions the venous walls come to rest (i.e. to equilibrium) at a point determined by the new subarachnoid pressure. There is, however, a distinct limitation of this process—the limitation of volume-dislocation which has been shown experimentally both in the dead and living animal. When this limit of volume-adjustment of the venous bed is reached the venous walls must remain in a "steady state", irrespective of the further alteration of the pressure on the outer sides.

For a century and a half the hypothesis that the skull and bony coverings of the vertebral canal form a rigid container for the central nervous system has occupied the attention of anatomists, physiologists, and neurologists. That a professor of anatomy at Edinburgh, Alexander Monro II, should have originated a doctrine of such basic significance in intracranial physiology should arouse the pride of every British anatomist. Monro (1783) ventured the hypothesis that the quantity of the blood circulating within the cranium must at all times be constant "as the substance of the brain, like that of the other solids of our body, is nearly incompressible" and as the brain "is

enclosed in a case of bone". Having no knowledge of the cerebrospinal fluid in its meningeal bed, though the publications of Haller (1757) and Cotugno (1779) preceded by a few years his own, Monro assumed a constant intracranial volume in which alterations in arterial volume were compensated for by reciprocal changes in the venous volume. Monro's original hypothesis was further developed by Kellie (1824), whose stimulating report is given in the first volume of the *Transactions of the Medical and Chirurgical Society of Edinburgh*, 1824. Kellie attempted experimental and pathological verification of the views advanced by Monro. Kellie's conclusions, based on observations in animals and in persons frozen to death, were that a state of bloodlessness did not exist in the brains of animals killed by bleeding, that the amount of blood in the cerebral veins was not affected by posture or by gravity, that congestion of these vessels was not found in those conditions in which it might well be expected, and that compensatory readjustments between the arterial and venous sides maintained a constant intracranial vascular volume. Kellie wrote "that in the ordinary state of these parts we can not lessen, to any extent, the quantity of blood within the cranium, by arteriotomy or venesection; whereas if the skull of an animal be trephined then hemorrhage will leave very little blood in the brain". With Kellie's apparent verification of Monro's hypothesis other workers applied the doctrine to pathological conditions in man, particularly in cases of apoplexy. In these studies an attempt was made to determine whether the cerebral haemorrhage was compensated for by decrease in the volume of the intracranial arterial and venous bloods. The thesis, which quite properly became known as the Monro-Kellie doctrine, was widely accepted and interest in it became profound.

The doctrine was necessarily modified when increasing knowledge of the cerebrospinal fluid developed from Magendie's first adequate description (1825) and his second comprehensive monograph (1842). Shortly after this second publication, Burrows (1846) questioned the accuracy of the hypothesis of fixed intracranial blood volume and introduced into the concept a third element—the cerebrospinal fluid. Burrows repeated many of Kellie's supposedly critical experiments relating to the effect of posture on the quantity of intracranial blood, and in his coloured plates is shown an apparent difference in these volumes in animals suspended post-mortem by the head or by the tail. Burrows placed great importance on the cerebrospinal fluid as the means of replacing blood lost through systematic haemorrhage, for he felt that exsanguination unquestionably diminished the quantity of intracranial blood. He was unable to decide whether "the space vacated under such conditions was filled with serum" (cerebrospinal fluid?) or was eliminated by "resiliency of the cerebral substances under decreased pressure"; but in this second phrase is contained the first suggestion that the volume of the brain may be altered by physiologic conditions. Summing up Burrows' contentions, it is clear that he was in general accord with the major thesis that the intracranial volume is at all times fairly constant—a thesis which accepts the view that the bony

containers of the central nervous system are rigid, preventing alteration in the total volume of the tissues and fluids within them.

Originally restricted to the cranium, the doctrine of a rigid container for the central nervous system with a fixed and constant capacity was necessarily extended to include the vertebral portion of the system. This modification was essential when it became generally appreciated that the cerebrospinal fluid of the spinal subarachnoid space communicated freely with that of the cerebral ventricles and cranial subarachnoid space (Key & Retzius, 1876). The vertebral arches were then looked upon as affording the requisite rigidity for firm suspension of the spinal dural sac. The Monro-Kellie doctrine thus came to be interpreted as the concept which viewed the entire central nervous system as being enclosed within a bony container (cranium and vertebral canal) of sufficient rigidity to secure constancy in volume of the intradural contents. Such an idea involved appreciation of the anatomical relationships within the whole cranio-vertebral system and was intimately related to the integrity of the skull and of the vertebral column whose relative rigidity was held to modify in an essential way the spinal dural component. In this modified form the Monro-Kellie doctrine of a rigid container of fixed volume has really afforded the basic hypothesis for all studies on the pressure and volume relationships about the nervous system.

As such a thesis necessarily affected all ideas of postural change in the pressures of the cerebrospinal fluid and of intracranial blood vessels, it is not surprising to find that shortly after Burrow's publication attempts were made to ascertain the truth of the important hypothesis by experimental methods. Many workers (Donders, 1851; Kussmaul & Tenner, 1859; and others) essayed by direct observations through a cranial window to secure evidence regarding the constancy or variability of the intracranial vascular volume; their methods, more reliable than observations on dead animals, did not permit control of all of the factors. The data presented by these workers hardly justified the conclusion of a variable intracranial blood volume, yet certain of these observations have bearing on the present consideration of the problem. Thus Ecker (1843) recorded in a trephined animal a marked diminution of the cerebral volume on division of the carotid arteries, thereby recalling attention to the function of the cranial vault in protecting the nervous system from the direct application of atmospheric pressure.

Many years later, Hill (1896), introducing more rigid methods of physiological control, concluded that "the volume of the blood in the brain is in all physiological conditions but slightly variable". Again here in London, Dixon & Halliburton (1914) studied the general problem of the Monro-Kellie doctrine by methods but slightly different from those employed by Hill. Basing their conclusions on the apparently great variations in intracranial pressures, particularly in the relation of the pressure of the cerebrospinal fluid to that in the torcular herophili, they asserted that "the cranial contents cannot any longer be regarded as a fixed quantity without the power of expanding or

contracting in volume". Such an assertion necessarily involved extreme modification of the doctrine, if not definite renunciation. The observations of Dixon & Halliburton indicated that unquestionably, within the physiologic limits established, variations in the pressures of the cerebrospinal fluid and of the cerebral venous blood could be affected without the exact correspondence reported by Hill.

My interest in this doctrine had been aroused many years before my work led me into direct contact with the hypothesis. During the course of an investigation to determine what agents, if any, would affect the volume of the brain, Weed & McKibben (1919 *a*, *b*) ascertained that the intravenous injection of solutions, the osmotic pressure of which differed from that of the blood, caused in the living animal outspoken alterations in the volume of the brain. It was shown by us that hypotonic solutions given intravenously markedly raised the pressure of the cerebrospinal fluid and increased the volume of the brain, while hypertonic solutions on similar administration caused lowering of the pressure of the cerebrospinal fluid and a corresponding diminution of the volume of the brain. With strongly hypertonic solutions, the pressure of the cerebrospinal fluid was frequently reduced to negative values (i.e. below atmospheric pressure), so that occasionally negative records of as great a magnitude as the previous positive readings were obtained.

It was apparent that these alterations in the cerebral volume and in the pressure of the cerebrospinal fluid, effected by the intravenous injection of solutions of various concentrations, were dependent upon the interchange of water and salts between blood and the nervous system with its cerebrospinal fluid. The negative pressures in the cerebrospinal fluid involved even more the consideration of the accuracy of the Monro-Kellie thesis. The experimental production of a pressure below atmospheric in the cerebrospinal fluid could be brought about only if the elasticity of the elements contained within the cranium and vertebral canal were exceeded, only if the elastic limits of the system were surpassed by the magnitude of the forces applied. Thus the great withdrawal of fluid from the nervous system, due to the strongly hypertonic solutions within the blood stream, apparently exceeded the elastic limit of the craniovertebral contents. Under these circumstances, the bony containers of the central nervous system came to serve as a rigid system, preventing the direct application of atmospheric pressure to the intradural contents.

This work on the effect of solutions of different concentrations was continued with the co-operation of Hughson (1921 *a*, *b*, *c*). Our first undertaking was the determination of the general systemic and intracranial effects of these solutions when given intravenously, and a second problem dealt with the Monro-Kellie hypothesis directly. We were able to show that the intactness of the cranial vault is essential for the production of negative pressures in the cerebrospinal fluid, following the intravenous injection of hypertonic solutions. With the cranial dura mater exposed directly to the atmosphere, negative pressures could not be obtained, even though marked shrinkage of the brain occurred.

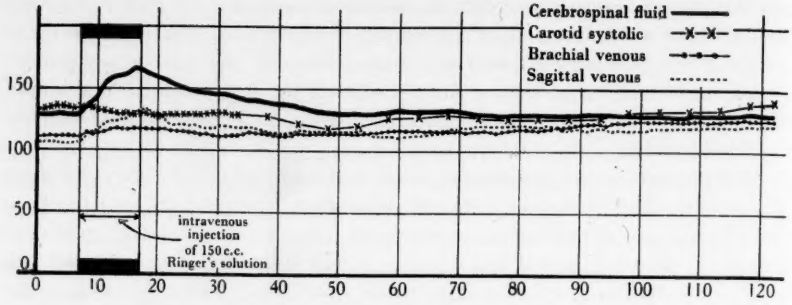

Fig. 6. Intravenous injection of 150 c.c. Ringer's solution.

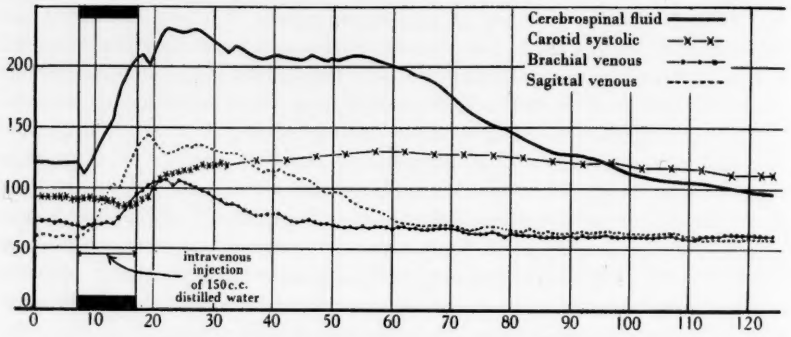

Fig. 7. Intravenous injection of 150 c.c. distilled water.

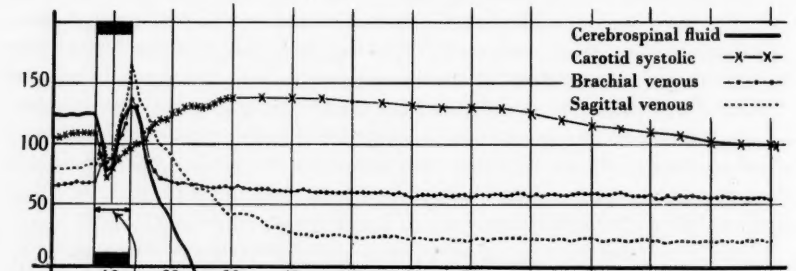

Fig. 8. Intravenous injection of 30 c.c. 30 % NaCl.

It was realized that these methods of demonstration were drastic in nature and that the means employed were such as to bring out maximum variations rather than the minute effects which might theoretically play a rôle in the normal physiological use of the bony containers of the nervous system. Our own findings bore a very definite relationship to the original observation of Kellie on the difference in intracranial blood volume in intact and trephined skulls on alteration of the animal's posture, and it was but a logical step to shift investigative interests to the problem of pressure-alterations about the nervous system as effected by changes in the position of the experimental animal.

First of all, it seemed obvious that the degree of rigidity of the bony cranium and of the vertebral column would determine in some measure such alterations of the pressure of the cerebrospinal fluid. Were the spinal dural tube so suspended within the epidural space that it could not collapse inward, were its fibrous architecture of such small elasticity that outward distensibility were hardly measurable, were the cranium with its adhering dura a rigid box-like container, and were the vascular regulatory mechanisms of the intradural contents so effective that no volume-change or dislocation of blood would occur?—if these suppositions were correct, no cerebrospinal fluid would be extruded from the needle on puncture in the horizontal position. Likewise if these suppositions were correct, the measurable pressures of the cerebrospinal fluid in the horizontal position would be reproduced in the vertical condition of a mammal, for this assumed rigidity of the containers of the nervous system would then be such as to prevent a dislocation of fluid from the uppermost portions of the system to the lowest. Such potential dislocations of the cerebrospinal fluid would be necessary for measurable alterations in pressure, even though in such positional change, increase in height of the vertical column of molecules imposed upon the lowermost portion would undoubtedly effect a pressure-change within the fluid itself.

The problem of the cerebrospinal fluid in relation to change in posture is related primarily to the potential pressure-changes as may be effected by the fluid-column in the subarachnoid space. Anatomically and physiologically this column of fluid is continuous, even in the meshes of the subarachnoid space. The first question necessarily is whether such a potential column exerts its full hydrostatic effect when the animal is abruptly tilted from the horizontal to the vertical position or whether the elasticity of the cranio-vertebral system is such as to prevent the action of the full column of fluid. The answer to this question would give some hint of the degree of protection of the nervous system from the effects of atmospheric pressure and some suggestion of possible physiological adjustments which might have taken place when man assumed the erect posture.

The need for an analysis of these potentialities in relation to the Monro-Kellie thesis has been met by a series of experiments involving tilting anaesthetized animals, from the horizontal to the vertical head-down and tail-down

positions (Weed, 1929). Most of these observations were carried out on dogs of uniform size and the findings in this animal may be considered to be typical, as the reactions in the cat, dog, and macaque are essentially similar. In these dogs the distance from the occipital protuberance to the last lumbar spine was in the neighbourhood of 400 mm.; this measurement may be taken to be that of the height of the column of fluid which might exert a hydrostatic effect in the two vertical positions. The tiltings from the horizontal to the head-down position caused increases in the pressure of the occipital cerebrospinal fluid which averaged 105 mm. of normal saline solution. In the contrariwise tiltings, from the horizontal to the vertical tail-down position, an average decrease of 74 mm. was recorded in the pressure of the cerebrospinal fluid, as measured in the occipital region. Changes in the carotid pressure (reflecting intracranial arterial pressure) were not of great significance during such positional alterations, but the head-down tiltings caused an average increase of 184 mm. in the pressure of the superior sagittal sinus, and the opposite positional change, an average decrease of 80 mm.—in each case changes of greater magnitude than in the cerebrospinal fluid itself. In the fluid itself, only one-third to one-fourth of the potential hydrostatic column was effective.

Another type of experiment having significance in our discussion had also been carried out (Weed & Flexner, 1932 *b*). In this the pressure of the cerebrospinal fluid was recorded in the occipital region and by lumbar puncture measurement was made in the lower spinal region. In the horizontal position the pressures in both manometers were the same, but when the dog was tilted to the vertical head-down position the fluid in the lumbar manometer ran in the animal's subarachnoid space, to be followed by air. Under these circumstances atmospheric pressure was directly applied to the cerebrospinal fluid throughout the lower spinal segment; the pressure-increase measured in the occipital manometer became approximately 185 mm. instead of the customary 105 mm. in the intact preparation.

These observations were followed by further experiments (Weed & Flexner, 1932 *b*; Weed, 1933 *a*, *b*) in which the cranial vault was largely removed, or in which laminectomy was performed throughout the lumbar and thoracic regions. With the pressure of the cerebrospinal fluid measured by an occipital manometer, both of these preparations exhibited normal pressures of the cerebrospinal fluid in the horizontal position. In those dogs in which the cranial dura mater was widely exposed to the atmosphere, the head-down tiltings gave pressure increases of the cerebrospinal fluid of the same extent as in the intact animal; but on tilting from the horizontal to the tail-down position, the pressure of the fluid decreased only 56 mm. on the average (74 mm. in the intact dog). On the other hand, the laminectomized animal showed, on the tail-down tiltings, a pressure-decrease of the same magnitude as in the intact dog; but on vertical head-down tilting the pressure of the occipital cerebrospinal fluid increased on the average 185 mm., as compared to 105 mm. in the intact dog. Of particular significance was the fact that in the laminectomized and

lumbar-punctured animals the increases of pressure on vertical head-down tiltings were identical with the pressure-increases in the superior sagittal sinus; the full effect of atmospheric pressure was operative through the lumbar needle and upon the exposed spinal dura mater. The increase in intracranial venous pressure was taken to be a reflection likewise of hydrostatic pressures exerted through the soft parts of the body.

The data from these experiments may be interpreted as demonstrating that the vertebral arches and cranium have a definite physiologic function in removing the central nervous system from the full effects of atmospheric pressure. They lead also to the assumption that within these bony coverings there are elements which lack rigidity, as an obvious dislocation of fluid occurs within the dural sac during such positional tiltings. To speak of such a lack of rigidity within the bony coverings is really another way of saying that the system possesses elasticity—an elasticity which allows a dislocation of fluid from one part of the subarachnoid space to another—not the maximal but a fractional dislocation, permitted by dilatation and compression of the intradural vascular bed, by inward collapse of the spinal sac with stretching of the epidural fibres and dilatation of the epidural venous plexus. The stretching of the spinal dura mater itself has been found to be so small as to afford a negligible expansibility.

Some of these questions had been advanced by Grashey (1892) in a monographic presentation of the hydrostatics of the cerebrospinal fluid. As a hypothetical exposition of certain phases of the problem of the potential modification of hydrostatic effects through the elasticity of the vascular components, Grashey's contribution has been of great value. A far simpler, purely physical representation of the theoretical relationships of the cerebrospinal fluid and its containing membranes was presented by Weed & Flexner (1935 *b*). The system was likened to a rigid tube, enclosed by elastic membranes at both ends and completely filled with fluid at atmospheric pressure. Such a model, in the vertical position, will have the level of atmospheric pressure midway between the two sagging membranes, if these membranes are of equal elasticity. In this vertical position a decrease in the elasticity of one of the membranes will shift the level of atmospheric pressure toward the more elastic membrane. Or again, the physics of the problem may perhaps be even more clearly illustrated by a single system consisting of a rigid central bar to which are attached two rigid discs; the walls of the system are formed by elastic membranes sealed to the outer edges of the discs. When filled with fluid at atmospheric pressure and placed in a vertical position, inward collapse of the membrane occurs in the upper half of the system and outward bulging in the lower.

Study of the pressure-reactions in such a physical system with the inward collapse and outward bulge of its membranes, and consideration of the data from the experimental animals lead to the conclusion that the explanation for the phenomena of positional pressure-changes in the cerebrospinal fluid un-

questionably rests on the dislocation of fluid coincident with change of the hydrostatic column. But the measurement of the fluid's pressure with an open-end manometer of small bore in the customary experiment permits an external dislocation of fluid—an obvious change in volume either into the manometer from the animal's subarachnoid space or from the manometer into the subarachnoid space. The influence of this factor of fluid-dislocation has been studied (Weed *et al.* 1932) in tilting experiments with measure of the pressure of the cerebrospinal fluid by the bubble manometer (recording pressure without external dislocation of fluid), and by manometers of various bores. The pressure-alterations in the cerebrospinal fluid, as recorded by the bubble manometer, were slightly greater than those in the open-end 1 mm. manometer; and as manometers of larger and larger bore were used the pressure-

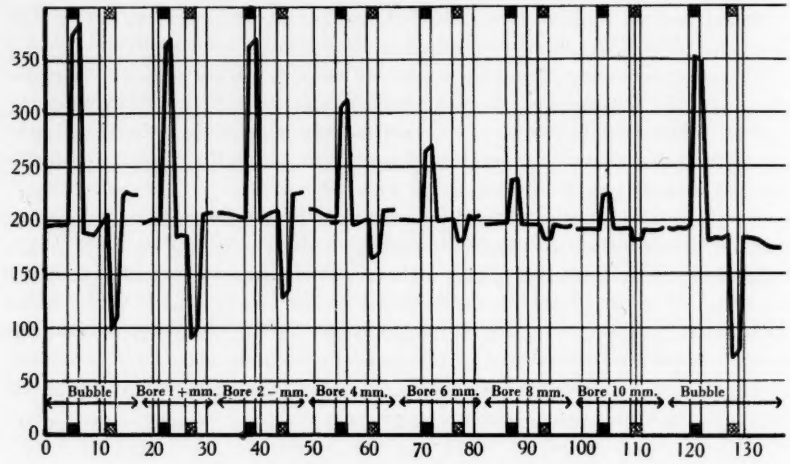

Fig. 9. Pressure-changes in cerebrospinal fluid in head up-tail down and head down-tail up positions, using manometer of larger and larger bore. (See text.)

changes became smaller and smaller in both positional tiltings. Determination of the fluid dislocated into or from these open-end manometers showed that a larger and larger dislocation of fluid occurred in the larger and larger manometers, though there was an obvious limit to the volume displaced.

This dislocation of fluid on tilting from the horizontal to the vertical position has led to the uncovering of a definite relationship between the pressure-differences and the volume-differences recorded in the cerebrospinal fluid (Weed *et al.* 1932; Weed & Flexner, 1932 *a*; Flexner *et al.* 1932; Flexner & Weed, 1933 *b*). This ratio, expressed in the term dV/dP, was found to be amazingly constant in the four mammals studied—dog, cat, macaque and a single chimpanzee. Its magnitude, approximately 0·17 in adult animals, is considerably smaller in immature animals and larger in the old; there is there-

fore a definite age-difference in the relationship. The ratio dV/dP is the same on determination from data obtained on head-down or tail-down tiltings—a fact of great significance in any discussion of the assumption of erect posture.

Table IV. *Derivation of dV/dP and E for macaque C* 30

	Head down					Tail down				
Manometer mm.	Pressure-change, C.S.F. cm.	Difference in pressure-change cm.	Volume dislocated c.c.	Difference in volume dislocated c.c.	dV/dP	Pressure-change, C.S.F. cm.	Difference in pressure-change cm.	Volume dislocated c.c.	Difference in volume dislocated c.c.	dV/dP
1	14·5	—	0·252	—	—	8·0	—	0·139	—	—
4	10·8	3·7	1·048	0·796	0·215	6·0	2·0	0·582	0·443	0·221
6	7·1	7·4	1·874	1·622	0·219	4·0	4·0	1·056	0·917	0·229
8	4·7	9·8	2·397	2·145	0·219	2·6	5·4	1·326	1·187	0·219
10	3·6	10·9	2·565	2·313	0·212	2·0	6·0	1·425	1·286	0·214
				Average	0·216					0·221

This ratio dV/dP is of course found in the usual physical formula for determining elasticity where elasticity is considered to be the stress divided by the strain—i.e. $E = dP/(dV/V)$. Using this formula, as had been done in a somewhat different way by Ayala (1923, 1925), and taking as the volume V the intradural contents, it was found that the elasticity of the intradural contents was of approximately the same magnitude in the four mammals tested. While the employment of this physical formula for determination of elasticity has been recently questioned (Pollock & Boshes, 1936), doubt as to the suitability of the formula has existed in the minds of my co-workers and myself for several years. The question essentially relates to the employment of any value within the nervous system as the total volume, or V, in the formula for the modulus. It is obvious that taking the intradural contents as V, figures of constant value are obtained, but it is doubtful whether the elasticity of the system can be determined with accuracy in terms of dynes.

The existence of such physiological elasticities within the cranio-vertebral system leads at once to consideration of the normal employment of these elasticities in life. The cranium and vertebral arches constitute a rigid container for the nervous system only when these elasticities are exceeded, yet they contain within their walls approximately the same total contents at all times. This relatively fixed volume is made up of brain, blood and cerebrospinal fluid. The volume of brain has been shown to be capable of experimental change; the volume of cerebrospinal fluid can be increased or diminished; and the volume of blood can surely vary. So with three variable elements comprising a relative fixed total volume, the idea of a reciprocal relationship, as first suggested by Monro and then by Kellie, becomes most logical. These workers did not restrict the reciprocal relationship to a diminution of cerebral blood-volume in cases of intracranial effusion of fluid, but applied it to the relationship between arterial and venous volumes within the cranium. The first com-

prehensive appreciation of the problem should be attributed to Burrows in his discussion of the Monro-Kellie doctrine; his portrayal of reciprocal volume-changes between blood and cerebrospinal fluid has remained accepted without essential modification. Recent investigations, based on observations through an intracranial window (Kubie & Hetler, 1928) or on chemical analyses (Weil *et al.* 1931), have demonstrated an increased intracranial blood volume after shrinking of the nervous system by intravenous hypertonic solutions—a reciprocal volume-adjustment by the blood for withdrawal of intradural fluid.

These reciprocal volume-adjustments apparently take place constantly in the normal life of the organism. They are probably of small amount in the quiet state, dependent here upon minor vascular adjustments or small changes in the osmotic pressures of the blood; but when positions of the vertebrate are changed by posture, the reciprocal adjustments are of considerable magnitude. In four-footed mammals, on abrupt tilting from the horizontal to the vertical positions, a demonstrable dislocation of cerebrospinal fluid occurs from the uppermost to the lowermost portions of the nervous system. This dislocation of cerebrospinal fluid is permitted and accompanied by local reciprocal volume-adjustments—the venous bed in the uppermost portion of the nervous system becomes dilated while that in the lowermost portion becomes compressed. The mechanism of adjustment in local volumes is apparently prompt and effective.

In the four-footed mammals studies (Weed *et al.* 1932; Weed & Flexner, 1932 *a*; Flexner *et al.* 1932; Flexner & Weed, 1933 *b*)—cat, dog, macaque and chimpanzee—the relationship between volume-change and pressure-change in the cerebrospinal fluid is the same when determined from data derived from head-down and tail-down tiltings. Two of these animals—the macaque and the chimpanzee—spend almost as great a portion of their lives in the erect posture as does man, and in these as in the other four-footed mammals there is no indication of the development of any physiological protection against the dislocation of cerebrospinal fluid on positional change of the body. If this deduction be correct, our inquiry shifts logically to the problems presented by the giraffe with its head held high above his spinal cord and by the bat which hangs suspended head-down. But no data from these highly specialized mammalian forms are as yet available, and we are therefore forced to proceed directly to comparable positional alterations in the pressures about the nervous system in man.

Many important reports, particularly by Zylberlast-Zand (1921), Pfaundler (1899), Ayer (1926), and Barré & Schrapf (1921), on differences in lumbar pressure of the cerebrospinal fluid in man in the prone and in the erect position, indicate that a comparable dislocation of fluid occurs in man as in the four-footed mammal on positional change. Ayer's finding of a small negative pressure on cistern puncture in man in the sitting position and Walter's (1929) deduction that in erect man the point of atmospheric pressure is in the mid-thoracic region, permit one to hypothesize that in this erect position man has

a negative pressure of not less than −400 mm. saline at the calvarium. And recent direct observations (Masserman, 1934, 1935) of lumbar pressures on tilting patients lead also to the conclusion that dislocation of cerebrospinal fluid from the uppermost to the lowermost parts of the nervous system occurs on positional change. The physiological mechanism in man seems therefore to be essentially the same as in the four-footed mammal: there was apparently no development of a special protective mechanism when man stood erect.

But has not our story of the cerebrospinal fluid given one suggestion of a protective mechanism within this rigid cranio-vertebral system? It was shown previously that alterations in the pressure of the cerebrospinal fluid have no effect on the pressure in the cerebral veins, even though the cerebral venous volume changes rapidly in compensatory fashion. The operation of this mechanism is apparently such that in the assumption of the erect position for instance, a marked lowering of the pressure of the cerebrospinal fluid in the cranium occurs, the cerebral venous volume there increases, but the cerebral venous pressure is unaffected by the decrease in cerebrospinal fluid pressure. Any decrease in the cerebral venous pressure under these conditions is the reflexion of change in the systemic venous pressure, effected by change in the hydrostatic columns of venous blood. Thus there exist local reciprocal volume-adjustments within the cranio-vertebral system on change in posture—not reciprocal pressure-adjustments. This physiological arrangement may possibly be the saving mechanism which allowed the four-footed horizontal mammal to become a vertical, erect organism, for in spite of an apparent dislocation of a long column of cerebrospinal fluid, with its accompanying pressure-changes about the nervous system, the intracranial venous system retains an effective pressure capable of returning blood to the systemic circulation. But was not there a great physiological hazard when the first vertebrate, with a horizontal nervous system, developed mobility of neck and head, so that the head could be raised above the rest of the nervous system, thus occasioning a dislocation of cerebrospinal fluid from the head-end of the organism and altering so markedly the pressure and volume-relationships about the neural axis? Fortunately nature saw to it that a rigid skull and vertebral column were provided: even to-day we should be thankful that we stand erect under the protection and aegis of a Monro-Kellie doctrine.

But these speculations and deductions could be continued almost indefinitely regarding this clear limpid fluid surrounding the nervous system. In a way I have attempted to give a somewhat connected story of the cerebrospinal fluid from its origin in the cerebral ventricles to its final pathway of absorption into the venous system. And in addition I have tried to touch upon some of the problems which arise in the fluid from the assumption of the erect posture by man. The story has not been in any sense a complete one but it has followed the rambles of one's research interests into various aspects of the problem for 20 years. It has been the tale of a certain

opportunism in research, but the tale is probably the more interesting because of the side-paths followed rather than the continued pursuit of a logical plan of investigation.

Over 20 years ago I asked myself this question: What actually does the cerebrospinal fluid do around the nervous system? Why is that fluid there and what function does it fulfill? Halliburton (1916) had asked himself the question and had concluded that it was "an ideal physiological saline solution". With that general statement no one working on the fluid can possibly disagree, but does it get us very much farther along the pathway of knowledge? We know that such a fluid-bed may protect the nervous system from certain shocks and traumata; we have evidence that the fluid carries off certain waste-products from the central nervous system, as is done by endothelial-lined lymphatic vessels in other parts of the body. We understand also that the fluid may serve a nutritive function for the delicate arachnoidea which is almost entirely devoid of a capillary network. We are aware, furthermore, that the cerebrospinal fluid may afford certain advantages for the animal organism in permitting partial floating or suspension of the nervous tissues in a fluid-bed. On the other hand, we have come to realize that the cerebrospinal fluid is capable of dislocation as the vertebrate changes posture, and for this dislocation there is no apparent compensation in the physiological mechanism. Is not this fluid disadvantageous as a water column when man stands erect? Or is not its chief function one of providing means of prompt reciprocal volume-adjustment when there occur changes in volume of one or another of the two remaining elements within this rigid container of the nervous system? Here perhaps is one of the real functions of the cerebrospinal fluid—a means of reciprocal volume-adjustment within a container which is largely protected from atmospheric pressure. But all of these speculations must remain as pure speculations and to-day we know so little more of the essential function of the fluid than we knew a quarter of a century ago. Yet we have become somewhat more certain as to how and where the fluid is produced, somewhat more certain as to how and where the fluid is returned to the venous system, somewhat more certain as to how and where the nervous system is protected by the three membranes and craniovertebral container. But there is still much to be learned about the meninges and cerebrospinal fluid—the problem must still be followed with equal regard for structure and function.

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THE EXOGENOUS CELLULAR CONTENT OF THE NEUROHYPOPHYSIS IN MAN UNDER NORMAL CONDITIONS

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The pars nervosa of the pituitary gland, or neurohypophysis, may be divided from the cellular point of view into endogenous and exogenous parts. The former consists of the cellular elements constantly present in the lobe, such as the pituicytes, whilst the latter comprises those cellular elements which have an origin outside the neurohypophysis and occasionally invade it. It is with these latter structures that this paper deals.

A comprehensive survey of the literature relating to this question shows that an invasion by basophil cells has been recognized for several years. Only the anatomical and histological aspects of the cellular invasion of the posterior pituitary lobe will be considered here, those considerations which have a clinical and pathological bearing being dealt with elsewhere.[1] Such histological features are the relation of the invasion to normal health, age and sex, the origin, fate and function of the invading cells, and the sites and levels in the lobe from which the invasion takes place.

LITERATURE

The first case of a basophilic invasion of the neurohypophysis was described by Thom (1901) in a woman of 90 years. Tolken (1912), Cooper (1925), Kiyono (1926), Guizetti (1927), Biggart (1935) and Rasmussen (1933) observed that such an invasion was more marked with advancing years. Vogel (1912), Dieckmann (1924) and Guizetti (1927) demonstrated that a basophilic invasion was present in young people but Guizetti considered that the cellular invasion in infants was not basophilic until the 7th year. An example of a truly basophilic invasion in a child of 30 hours is shown from the writer's series (Pl. I, fig. 1).

Herring (1908) considered that the basophil cells invading the neurohypophysis were derived from the pars intermedia and that they were broken down to form "hyaline bodies", which were found in the glial spaces, and these bodies constituted the internal secretion of the posterior lobe. Halliburton *et al.* (1909) and Dieckmann agree with Herring, but Dieckmann described the process in greater detail and indicated the preliminary arrangement of the cells into alveoli prior to the actual invasion. Lewis & Lee (1927) showed that the

[1] Wade, H. J. "The exogenous cellular content of the neurohypophysis in man under pathological conditions." *Brain*, 1938 (in preparation).

tubulo-racemose glands seen in the pars intermedia of children, which up to the age of 4 years communicate with the cleft, and the basophil cells of the pars nervosa were derived from the pars intermedia. Several examples of glandular remains are shown in the present series (Pl. I, fig. 2) and the pars intermedia cells are readily distinguished by their columnar shape, finely granular cytoplasm and a slight affinity for the acid stain. Bucy (1932) considered that the invading cells were derived from the pars anterior and that they could not be the source of the "hyaline bodies" described by Herring because the latter are found in cattle in which there is no pars intermedia and no invasion of the pars nervosa. These bodies are also found in the infundibulum where no basophil cells have been seen and he concluded that they must arise from the degeneration of those nerve-endings described by Tello (1912). Cushing (1933) considered that the invading basophil cells were derived from the pars intermedia and that they were responsible for the production of pituitrin. He described an arrangement of these cells, even in the inactive or chromophobe state, into vesiculi which contained colloid; these cells became ripened into basophil cells, invaded the posterior lobe and gradually lost their staining affinities to form "hyaline bodies" which, he considered, represented pituitrin on its way, via the infundibulum, into the third ventricle. Cowdry (1934) upheld an origin of the invading cells from the pars intermedia. Gersh & Tarr (1935) and Tarr & Geiling (1934) showed that the "hyaline bodies" were not derived from the pars intermedia or the invading cells and that they were artefacts. Butt & Van Wart (1935) and Rasmussen considered that the invading cells were derived from the pars intermedia. It will be seen that the weight of the evidence is in favour of an origin of these invading cells from the pars intermedia.

METHOD OF APPROACH AND TECHNIQUE

The pituitary glands were obtained from post-mortems performed in both voluntary and municipal hospitals as well as those performed by several police surgeons. The control glands were obtained from street and industrial accidents. The possibility of these exhibiting pathological conditions, however, was not overlooked. Such material was immersed in 5 per cent formol saline on removal. The usual process of preparation for paraffin sections was employed, with the exception that a longer period of time was allowed in methylated spirit to enable a clean dissection of the capsule of the posterior lobe to be made from the back of the sella turcica. The intact removal of this capsule was in many cases very difficult, particularly in patients over 50 years, and instructions were given that the posterior clinoid processes were desired intact. Serial sections of the glands were made from below upwards at 10μ, and every tenth section mounted. Some 3500 sections were obtained in this way and stained by the Haemalum and Eosin and Mallory Connective Tissue (phosphomolybdic acid) methods.

METHOD OF CLASSIFICATION OF DEGREES OF INVASION

(Pl. I, figs. 3 and 4)

The review of the literature shows that a basophil invasion of the posterior lobe of the hypophysis does occur. Authorities differ in their interpretation of the numerous factors as to the origin, function, and relation of these cells to age, sex, and disease. Each worker has described his own interpretation of the degrees of invasion with terms such as "slight", "moderate", etc. In the absence of any accepted method of classification, it is impossible to compare what one worker considered a "moderate" invasion with that of another. It is, therefore, necessary that some method of estimating the degree to which the invasion occurs be evolved so that results may be classified and accurately compared. The first method would be one of counting the individual basophil cells in the neurohypophysis so that the investigation would be accurate and truly quantitative. This is a most tedious task and it was found to be impracticable because of the close apposition of the invading cells to one another and the difficulty experienced in judging when a basophil cell had ceased to be a true cell and had become a shapeless mass. These difficulties were encountered even when sections 5μ in thickness were examined.

An arbitrary method of classification was evolved by the writer based on the microscopic fields of vision, using a $\frac{2}{3}$ in. objective in association with a No. 2 eyepiece. The section in each gland which showed the greatest degree of invasion was chosen for classification. The main drawback to this method is that the maximal invasion is only represented in a transverse plane and it does not assess a concomitant invasion so accurately in a longitudinal direction. In order to overcome this difficulty one would have to cut longitudinal and transverse sections of the same gland, a procedure which is impracticable. Four groups were thus classified:

Group 1. *Absent invasion.* No true invasion of the pars nervosa was seen in this group which includes those cases where a few individual cells were seen to enter the lobe at various levels as well as those cases where no basophil elements were seen in the lobe. Such a migration of a few cells into the neurohypophysis in no sense amounts to an invasion.

Group 2. *Low grade invasion.* Here a true invasion is seen. Columns of basophil cells enter the neurohypophysis from the juxtaneural zone and spread inwards along the connective tissue trabeculae of the pars nervosa. The total cellular content of the lobe in the section which showed the greatest degree of invasion is less than one-quarter of the microscope field at this magnification.

Group 3. *Moderate invasion.* The total basophil content of the neurohypophysis in the section which showed the greatest degree of invasion is greater than one-quarter but less than one-half of the microscope field at this magnification.

Group 4. *High grade invasion.* The total basophil content of the neurohypophysis in the section which showed the greatest degree of invasion is

greater than one-half but no greater than three-quarters of the microscope field at this magnification.

A further group (5) may be described in which the total basophil content in the section showing the greatest degree of invasion is greater than three-quarters of the microscope field at this magnification. However, only one example of an invasion greater than group 4 was obtained in the seventy-five hypophyses which were examined.

The above original classification has worked exceedingly well in the present series. It has the advantages of simplicity and rapidity and there is less margin of error in this method than in those which involve the tedious and difficult task of enumerating the individual cells.

SUMMARY OF FINDINGS IN RESPECT OF AGE AND SEX AND DEGREE OF INVASION

Seventy-five pituitary glands were available for microscopical examination and of these seventeen were in the "control" group and fifty-eight fell into the "pathological" class. Classification of the control glands according to age, sex and degree of invasion showed that there was a tendency towards an increased invasion with advancing years, and that the male neurohypophysis exhibited a greater degree of basophil invasion than the female. These findings were borne out on classification of the "total" and "pathological" glands in the same way and the latter suggested that arterial disease and renal fibrosis may be responsible for the increased basophil content in advancing years. Two cases of invasion were seen in children under the age of 10 days. There can be no agreement with Guizetti because his statements that the invading cells do not undergo a basophil change until the 7th year are not substantiated in this series (see Pl. I, fig. 1).

List of specimens

M. = male; F. = female; B.P. = blood pressure, systolic first

No.	Sex and age	Final diagnosis	B.P.	Group
12	M. 24	Lobar pneumonia, grey hepatization	120/80	1
14	F. 52	Pontine haemorrhage, chronic interstitial nephritis, atheroslerosis	280/140	1
18	F. 46	Street accident, fractured skull	—	1
21	M. 8	Purulent pericarditis	105/55	1
40	F. 44	Accidental death	—	1
59	F. 43	Accidental death	—	1
65	F. 1½	Pneumonia	—	1
67	M. 45	Accidental death	—	1
68	M. 22	Accidental death	—	1
69	M. 58	Bronchopneumonia	150/90	1
74	F. 52	Essential hypertension	250/130	1
76	F. 40	Cardiac failure, essential hypertension	280/150	1
77	M. 1 day	Pulmonary atelectasis	—	1
79	M. 52	Chronic bronchitis, syphilitic aortitis	160/80	1
82	M. 54	Accidental death	—	1
83	F. 65	Cerebral atheroma	200/150	1
84	M. 48	Monocytic leukaemia	140/90	1

List of specimens (cont.)

No.	Sex and age	Final diagnosis	B.P.	Group
86	M. 35	Chronic fibroid phthisis	130/90	1
89	F. 8 days	Haemolytic anaemia	—	1
90	F. 43	Essential hypertension, left heart failure	260/140	1
91	F. 64	Cerebral haemorrhage, atherosclerosis, chronic interstitial nephritis	200/100	1
92	F. 64	Accidental death	—	1
7	F. 68	Chronic interstitial nephritis, coronary thrombosis	270/190	2
17	M. 51	Lobar pneumonia	140/90	2
25	M. 2 days	Congenital pulmonary stenosis	—	2
29	F. 79	Carcinoma gall-bladder	160/100	2
31	F. 23	Accidental death	—	2
32	M. 31	Bilateral adrenal atrophy, Addison's disease	90/50	2
33	M. 41	Essential hypertension	240/130	2
35	F. 77	Atherosclerosis, chronic interstitial nephritis	260/140	2
37	M. 64	Atherosclerosis, chronic interstitial nephritis	240/140	2
42	M. 54	Accidental death	—	2
46	M. 55	Syphilitic aortitis, cardiac failure	150/45	2
47	F. 47	Hypostatic pneumonia	150/95	2
48	F. 58	Essential hypertension	240/120	2
51	M. 42	Accidental death	—	2
54	M. 56	Bronchopneumonia, right heart failure	140/90	2
55	M. 50	Accidental death	—	2
56	M. 35	Miliary tuberculosis	130/85	2
58	M. 40	Essential hypertension	260/150	2
66	F. 46	Chronic glomerulonephritis	260/150	2
70	M. 47	Lobar pneumonia	140/80	2
73	F. 61	Atherosclerosis, chronic interstitial nephritis	270/160	2
81	M. 10 days	Atresia oesophagus, tracheo-oesophageal fistula	—	2
85	M. 69	Atherosclerosis, chronic interstitial nephritis	260/160	2
88	M. 27	Cervical caries, compression paraplegia	130/85	2
93	M. 39	Addison's disease	95/60	2
4	M. 60	Cirrhosis liver, portal obstruction, chronic interstitial nephritis	260/170	3
5	M. 48	Essential hypertension	250/160	3
6	M. 33	Chronic glomerulo-nephritis, endarteritis of coronary arteries	240/150	3
9	M. 48	Bronchopneumonia, pulmonary embolism	140/90	3
10	M. 23	Tuberculosis of hip	125/90	3
11	M. 62	Ruptured aneurysm aorta, syphilitic aortitis, chronic interstitial nephritis	190/100	3
13	F. 12	Tuberculous meningitis	150/90	3
15	M. 61	Carcinoma bronchus	160/90	3
22	M. 69	Atherosclerosis, chronic interstitial nephritis	250/140	3
24	M. 75	Accidental death, arteries equivalent to age	—	3
36	M. 51	Lobar pneumonia	150/100	3
38	M. 33	Accidental death	—	3
39	F. 64	Atherosclerosis, chronic interstitial nephritis	260/150	3
44	M. 50	Atherosclerosis, chronic interstitial nephritis	200/110	3
45	M. 73	Tuberculous bronchopneumonia, enlarged prostate	160/90	3
52	M. 63	Carcinoma stomach	150/100	3
53	M. 50	Lobar pneumonia, essential hypertension, left heart failure	250/140	3

List of specimens (cont.)

No.	ex and age	Final diagnosis	B.P.	Group
71	F. 42	Myocarditis, cystitis	140/90	3
72	M. 59	Accidental death, arteries equal to age	—	3
78	F. 55	Accidental death	—	3
87	M. 64	Atherosclerosis, chronic interstitial nephritis	265/170	3
80	M. 58	Atherosclerosis, chronic interstitial nephritis	290/160	3
57	M. 40	Lobar pneumonia	135/90	4
61	M. 60	Carcinoma bronchus	140/90	4
62	M. 62	Carcinoma bladder	160/90	4
64	M. 39	Chronic glomerulonephritis, cerebral tumour	270/160	4
75	M. 60	Carcinoma bronchus	145/95	4
50	M. 66	Chronic glomerulonephritis	290/150	4 (++)*

* The only example which could be classified as Group 5.

ORIGIN AND FATE OF BASOPHIL CELLS IN THE NEUROHYPOPHYSIS

(Pl. I, figs. 5 and 6)

Two theories exist as to the origin of the basophil cells of the neurohypophysis. The first is that they arise from the pars anterior. If this theory is to be accepted why do the other cells of the adenohypophysis not equally participate in the invasion? Again the majority of sections show eosinophil cells of the anterior lobe on the other side of the boundary zone. Colloid substance and cleft-remains intervene between the two lobes in many cases and this fact, together with the observation that the invasion is taking place while the juxtaneural fibrous tissue is developing most rapidly, indicates the untenability of this theory. The other theory is that these cells arise from the pars intermedia and this is upheld by many authorities, particularly Rasmussen and Cushing. The remains of the pars intermedia vary to a great extent in man and these may consist of a cleft or part of a cleft lined by low columnar cells with slightly granular and faintly eosinophilic cytoplasm or scattered remains of ducts and tubular glands may be found in the juxtaneural zone. The process of basophil cell formation begins with the segregation of small portions of the primitive cleft to form locules or alveoli of cells which, together with a few ducts and tubular glands, may be seen in the fibrous tissue of the juxtaneural zone. Increase in the fibrous tissue causes a further segregation. Such cells are faintly eosinophilic and gradually assume a chromophobe tint and change to basophil cells. Such cells, in all stages of basophilic maturity, may be seen in alveoli in the juxtaneural zone. Invasion takes place from these alveoli along the connective tissue trabeculae of the neural lobe and these cells gradually become more deeply basophilic and brown pigmented areas appear in their cytoplasm. This is followed by loss of the cell membrane and a change into a homogeneous mass. These masses are frequently seen lying in the neuroglial network and a darker ring in the centre of the mass indicates the remains of

the nuclear chromatin. Such masses were observed by Cushing and called "cell ghosts". Later, when all remains of cellular structure have disappeared, these structures form some of the so-called "Herring hyaline bodies". The writer considers that the name "hyaline" should be discontinued in this respect as these bodies are constantly granular. He does not, however, accept the belief that these bodies are related to the hormone of the lobe but concurs with the findings of Gersh & Tarr that these structures are artefacts and represent part or all of the diffused protein of the lobe without any relation to pituitrin, but he believes that the basophil cells contribute in part to their formation. The probability that the basophil cells were responsible *in toto* for the production of the "hyalin emasses" has recently been disproved. Tarr & Geiling (1934) demonstrated the presence of these bodies in the whale and chicken which are without a pars intermedia and Gersh & Tarr (1935) have shown that they are more numerous in fresh specimens and are decimated in the process of fixation. This explains the lack of correspondence between the degree of invasion and the number of "Herring bodies".

Numerous examples of altered pars intermedia cells (chromophobe and faintly basophil), which are seen lying in alveoli in the juxtaneural zone, show that the change from pars intermedia cell to posterior lobe basophil cell is entirely normal in occurrence. This suggests the necessity for some stimulus to produce, not only a greater degree of production of such basophil cells, but also to cause the invasion to take place. No relation was found between the number of invading cells and the degree of pars intermedia remains (cf. Cowdry) and the writer suggests that the activity of these remains is the important factor and not their actual number.

THE QUESTION OF AN EOSINOPHILIC INVASION

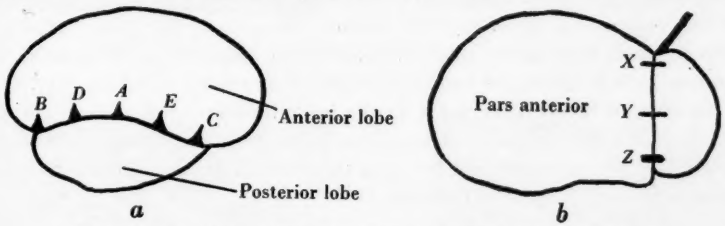
One case of an eosinophilic content of the neurohypophysis was encountered in the present research, in which ill-defined eosinophil cells and shapeless eosinophilic masses were seen in the extreme lateral edge of the pars nervosa on one side, while similar cells of the pars anterior packed the interstices on the other side of the juxtaneural zone. This phenomenon was observed in nine serial sections and the demarcation into cells was practically non-existent. Nothing in the nature of an invasion was taking place and these structures were lying amongst normal basophil cells in the neurohypophysis. In this area the fibrous tissue between the two lobes was absent and no remains of the primitive cleft were seen. The appearance presented was one of a fortuitous invasion of a few eosinophil cells from the adenohypophysis into the neurohypophysis, due to lack of development of the normal fibrous tissue and the absence of cleft-remains and colloid. These cells were not invading the lobe but were simply lying on their wrong side of the juxtaneural zone. No undue importance was attached by the writer to this and in no measure does it affect the theory and process described above in the discussion of the origin of the

invading basophil cells, since in all the work that has been done on the basophil content of the neurohypophysis but three observations of an eosinophil content have been described. Two cases were described by Tolken and Lewis and Lee and Orlandi mention its occurrence. Further, in cases where the distinction between the two lobes is ambiguous and scanty, it is possible that a few cells may pass into the pars nervosa from the pars anterior. This applies equally to the chromophil and chromophobe cells of the adenohypophysis and depends upon the nature of the cells found on the pars anterior side of the juxtaneural zone at the level examined. These structures are usually eosinophil cells and an explanation of this rare phenomenon is available.

The differences which exist between the basophil cells of the two lobes may be summarized as follows: the basophil cells of the neural lobe are not so vacuolated but show a greater degree of pigmentation than their counterparts in the anterior lobe. The appearances suggest that the latter is a senile change as it is constantly seen prior to their disintegration. Initially the posterior lobe basophils are smaller than those of the anterior lobe but become larger as they mature. Both types of cell are faintly granular and, as far as can be seen from the present collection of slides, the nuclear configuration is the same although no special nuclear staining methods were employed.

SITES AND LEVELS OF INVASION

The examination of serial sections in this research has demonstrated that there are five possible sites along the juxtaneural zone from which invasion of the pars nervosa takes place (Text-fig. 1). One is situated centrally, two at each



Text-fig. 1. *a*, showing sites of invasion; *b*, showing levels of invasion.

lateral extremity of the zone and two midway between them (Text-fig. 1 *a*). When the invasion is of the group 4 type, extension of these foci causes a more or less continuous linear arrangement of basophil cells along the juxtaneural zone. Careful examination of serial sections, and here the great importance of serial sections in this type of work may be stressed, shows that in the main, the higher grades of invasion take place from five such sites. Where the invasion is less marked, three sites are seen, one centrally and two laterally (Text-fig. 1 *a*, *A*, *B* and *C*). All the invading cells are not seen arising equally from these sites in any one section and as the process is traced from below upwards in the gland, first one site and then another is at its maximal stage of production, but

when the gland as a whole is considered, the description of these sites of invasion is accurate. Another site of invasion was observed in gland No. 50 (group 4++). Here a massive invasion was found occurring from the normal sites and in addition true basophil cells were seen streaming from the capsule of the neural lobe in a centripetal direction. It is in this situation that the pars intermedia-remains in the capsule of the neurohypophysis are sometimes situated. Such an invasion probably takes place in response to an excessive stimulation which produces an overaction of the pars intermedia-remains wherever they may be found, even in the peripheral capsule of the neurohypophysis. No previous example of such a "peripheral" invasion can be found in the literature examined and while it supports the description of the origin of the invading cells it also supports the belief in a rudimentary capsule derived from the pars intermedia which may enclose the posterior lobe.

Guizetti described four levels in the gland at which basophil elements may invade the pars nervosa; two are situated midway between the caudal and cephalic extremities and two at the caudal extremity (one on each side of the midline).

Many workers have performed microscopical examinations of the neurohypophysis but without the use of serial sections. Such investigations are totally useless. The cutting of so-called "representative slivers" at three levels (Spark) may show a few invading basophil cells but what evidence can that be for accurate classification when the invasion varies as one proceeds from the caudal to the cephalic extremities? Such findings are barely qualitative and in no sense quantitative.

Considerable variations in the degree of invasion are found from the above sites as one passes from the caudal to the cephalic extremity. The most constant level encountered is situated at the caudal extremity of the juxtaneural zone, a less constant level is seen midway between the two extremities and the least common level is found just below the cephalic extremity (Text-fig. 1 *b*). There are no sites or levels of invasion in the infundibulum. Such levels are most definite in the higher grades of invasion, but very few sections failed to show a few wandering basophil cells between the two extremities. These findings are not in accord with those of Guizetti.

SUMMARY

1. Ninety-three pituitary glands were obtained of which seventy-five were suitable for detailed examination.

2. The pars intermedia is present in man to a variable extent and from these remains the basophil cells of the neurohypophysis are developed.

3. Eosinophil cells of the adenohypophysis may be found very rarely in the neurohypophysis. This heterotopic finding is discussed.

4. A new classification of the degrees of basophil invasion is described.

5. There are more basophil cells in the male neurohypophysis.

6. The basophilic invasion is seen at five sites and three levels in the neurohypophysis.

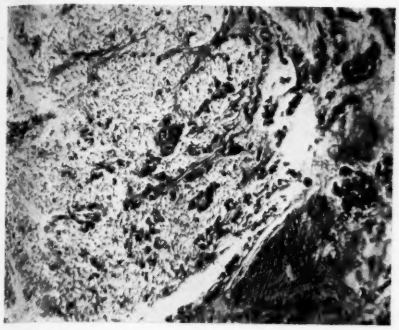


Fig. 1.

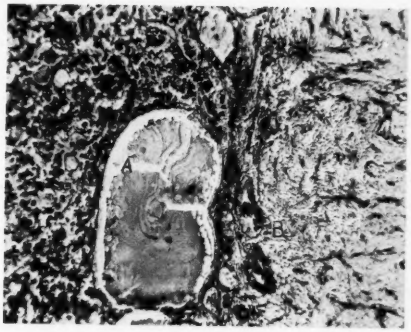


Fig. 2.

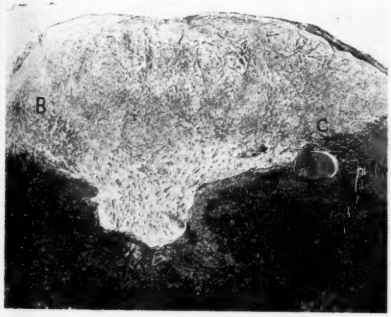


Fig. 3.

Fig. 4.

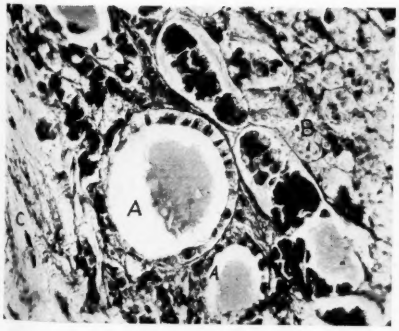


Fig. 5.

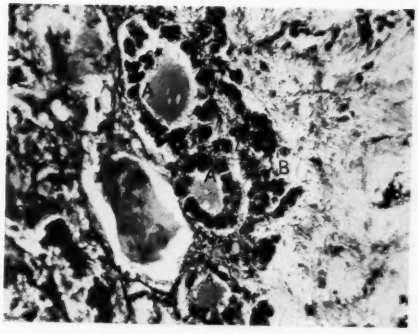


Fig. 6.

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7. The invasion of the neurohypophysis by basophil cells is a normal occurrence and may reach considerable proportions in perfectly healthy subjects. There is an indefinite relation to advancing age and it is suggested that this aspect is related to arterial disease and renal fibrosis.

ACKNOWLEDGEMENTS

I am indebted to Prof. J. S. B. Stopford, Prof. Donal Sheehan and Dr E. R. A. Cooper for their interest and advice in this research and to Mr H. Gooding for the microphotography.

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EXPLANATION OF PLATE I

Fig. 1. Microphotograph showing invasion of the neurohypophysis in a child aet. 30 hours by the true basophil cells.

Fig. 2. Microphotograph showing pars intermedia tubules in the neurohypophysis (*B*) and a large Rathke cyst (*A*) lined by typical pars intermedia epithelium.

Fig. 3. Microphotograph showing a group 2 invasion of the neurohypophysis from two sites (*B* and *C*).

Fig. 4. Microphotograph showing group 4 + + invasion of the neurohypophysis by basophil cells. Note oedematous appearance.

Fig. 5. Microphotograph of juxtaneural zone showing (*B*) adenohypophysis, and (*C*) neurohypophysis with pars intermedia tubules at (*A*). Stages in the differentiation of the cells lining these tubules into basophil cells are well shown.

Fig. 6. Microphotograph of juxtaneural zone showing (*A*) loculi of basophil cells containing colloid material, and (*B*) invasion of the neurohypophysis by basophil cells derived from the loculi.

A TECHNIQUE FOR OPERATIONS ON THE HYPOTHALAMO-HYPOPHYSIAL REGION OF THE RABBIT

By G. W. HARRIS

Marmaduke Shield Scholar, Department of Anatomy, Cambridge

AND GR. T. POPA

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Numerous operations have been described for reaching the hypophysial region, the routes suggested varying with the type of animal used. We shall not attempt to give here an account of the buccal, nasal, temporal, parapharyngeal, orbital or sphenoidal routes which have been elaborated, for good reviews of these may be found in various textbooks of endocrinology and surgery (Aschner, 1912; Biedl, 1913; Blair Bell, 1919 and Hirsch, 1929). The thesis of Popescu (1934) and Frasin (1935) contain lists of references to many relevant papers under the headings, "Hypophysectomie", "Chirurgie de l'hypophyse", and "Extirpation de l'hypophyse".

It was of importance, for the purpose of some of our experiments, to find a method of operating on the hypophysial region of the rabbit. For hypophysectomy in this rodent, White (1934), and Firor (1933), have described buccal and orbital routes, respectively. After attempting to section the pituitary stalk by both these operations, the conclusion was drawn that a method giving a better exposure of the hypothalamo-hypophysial region was required, leading to the investigation of the temporal route, which is described below.

OPERATIVE TECHNIQUE

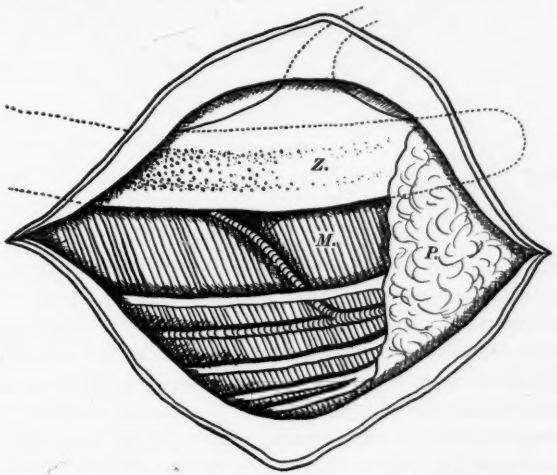
The skin over the lateral aspect of the head and face is first shaved and the animal is then anaesthetized. The most satisfactory anaesthetic was found to be ether administered through a tracheal cannula. At the completion of the operation, the trachea and pharynx must be carefully cleaned from mucus before suturing the trachea with one or two stitches.

The first stage of the operation (Text-figs. 1 and 2) deals with the removal of part of the zygomatic arch.

The skin incision should be made over the posterior two-thirds of the zygomatic arch, to the root of the ear. The cut should be made firmly and deeply through the superficial fascia as well as the skin, for if these two structures are not reflected together, and they are easily separable, gangrenous changes in the skin are likely to supervene later. The periosteum over this

region of the zygomatic arch is then reflected, particular care being taken not to damage large vessels at the anterior and posterior margins of the masseter muscle, which are branches of the external carotid artery (a. occipitalis,

Text-fig. 1. Exposure of zygomatic arch. *M.* upper and posterior part of masseter muscle; *P.* parotid gland; *Z.* zygomatic arch. Note also, the transverse facial vessels, parotid duct, and branches of the facial nerve.



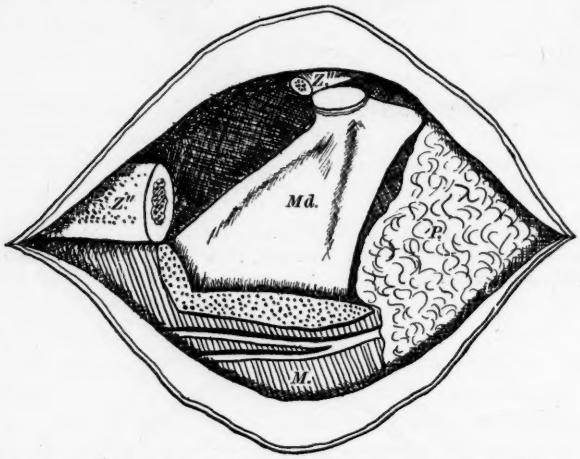
Text-fig. 2. Operative field, as shown in Text-fig. 1 enlarged.

a. temporalis superficialis and a. maxillaris interna) and of the external jugular vein (v. facialis anterior and v. facialis posterior).

The zygomatic arch must next be cut through with bone forceps, first at the junction of the anterior one-third with the posterior two-thirds and then at its

posterior extremity, the masseter muscle still being attached. (If the masseter muscle is separated before the arch is cut, the masseteric vessels will bleed, and cannot be ligatured or compressed until the arch is removed.) The masseter muscle is then cut at its origin and the piece of zygoma removed. The remains of the posterior end of the zygomatic arch is then entirely removed with bone nibblers, thus exposing the temporo-mandibular joint. Haemorrhage is likely to occur at this stage from the masseteric vessels and from the vessels around the jaw joint. This is unavoidable but can be controlled by pressure.

The second stage (Text-fig. 3) is the removal of the upper one-third of the ramus of the mandible.



Text-fig. 3. Removal of zygomatic arch and part of masseter muscle. *M.* masseter muscle; *Md.* superior half of the ramus of the mandible; *P.* parotid gland; *Z.,'' Z.''* two cut ends of zygomatic arch.

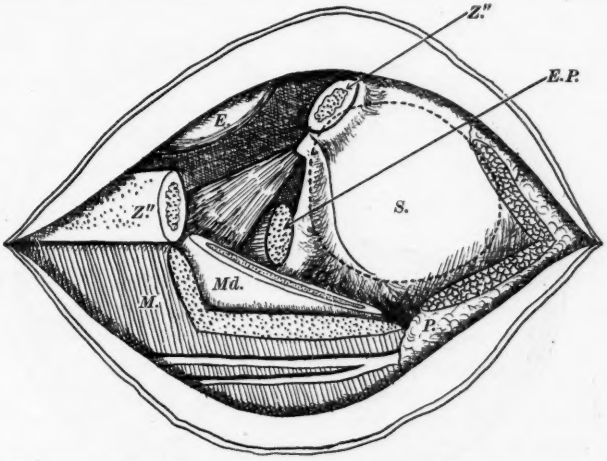
The masseter muscle overlying this area of bone is cut through cleanly and laid aside on a piece of aseptic towelling for use as a muscle compress. The periosteum is removed from the exposed bone surface. The ramus is lifted gently, and the mandibular articular surface of the temporo-mandibular joint is separated from the mandible by gentle pressure; it is then possible to reflect the periosteum further down the medial aspect of the ramus. Gauze swabs may then be pushed underneath the ramus, tending to compress the underlying structures and raise the bone so that it may be cut across more easily with the bone forceps.

The third stage (Text-fig. 4) consists of exposing and removing part of the squamous temporal bone.

The pterygoideus externus muscle is clipped away piece by piece and the pterygo-maxillary fossa is gradually cleared. The haemorrhage met with in this region may be quite considerable, but with practice and care can be checked

by compression with gauze pads, or better, pieces of muscle. The area of the skull to be exposed is that part lying superior to the pterygoid process and posterior-inferior to the structures in the orbit. It is advisable not to dissect too deeply into the pterygomaxillary fossa for here lies the internal maxillary artery with its branches, and the main divisions of the trigeminal nerve.

Before attempting to open the skull it is important that all bleeding and oozing should be stopped. While awaiting the cessation of the haemorrhage, the opportunity should be taken of injecting about 10 c.c. of 15 % saline into the marginal vein of the ear; this will cause a decrease in the volume of the brain, which will be of great advantage in the later stages of the operation.



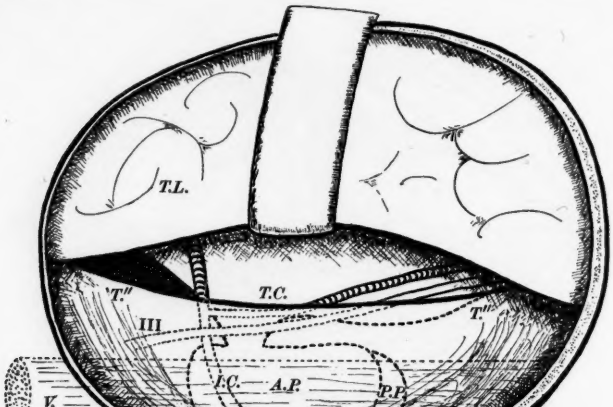
Text-fig. 4. Exposure of the skull. *E.* structures in the orbit; *E.P.* external pterygoid muscle; *M.* masseter muscle; *Md.* cut end of ramus of mandible; *P.* parotid gland cut; *S.* surface of skull; *Z.'*, *Z.''* two cut ends of zygomatic arch. The area of skull removed is shown by the dotted line.

The skull is attacked in the temporo-sphenoidal region, just anterior to the tympanic bulla and superior to the pterygoid process, where the bone is very thin. A small piece of this delicate plate of bone may be removed by gently tapping a scalpel held with its point at a very oblique angle to the surface of the skull, until a spicule of bone is displaced. This opening is then enlarged with a small pair of bone nibblers, exposing the dura mater covering the temporal lobe of the brain. The dura mater is then incised with a fine iridectomy knife and reflected.

The fourth and final stage of the operation now begins (Text-fig. 5), consisting of the exposure of the hypothalamus and hypophysial region.

The temporal lobe of the brain is very carefully and slowly retracted and at the same time the cerebrospinal fluid is absorbed with fine swabs. At this stage it is advantageous to use a head-lamp, for the field of operation is now

deep and small. On full retraction of the brain the edge of the tentorium cerebelli comes into view, above which may be seen the hypothalamus. The essential landmark in this region is the small internal carotid artery which may be easily seen if the retraction has been properly performed, on account of the colour contrast of the red artery against the white background of the brain substance. Its position may also be gauged by palpation of the anterior clinoid process, the posterior end of which marks the point of appearance of the artery above the tentorium. Postero-medial to the artery lies the tuber cinereum and the hypophysial stalk. Further posterior may sometimes be seen the oculomotor nerve, disappearing from view. Thus the whole region is laid bare before the operator.



Text-fig. 5. Retraction of temporal lobe of brain. *A.P.* anterior lobe of pituitary; *I.C.* interna carotid artery; *P.P.* posterior lobe of pituitary; *T.″* tentorium cerebelli; *T.C.* tuber cinereum; *T.L.* temporal lobe of brain; III. oculomotor nerve; V. trigeminal nerve.

The instrument that was used in our experiment for making lesions in the pituitary stalk consisted of a small piece of razor blade, ground to the required shape and mounted at the end of a curved probe.

On completion of the operation, the brain is allowed to fall gently back into place, the dura mater replaced as far as possible over its surface, and then the skin sutured with Michel's clips and painted with iodine and colloidon-ether solution.

CRITICISM OF THE METHOD

The drawbacks of this technique may be divided into two groups, operative and post-operative complications.

There are two main operative difficulties, haemorrhage and damage to the brain. All the haemorrhage met with may be controlled by pressure from

gauze pads or pieces of muscle, except the intracranial bleeding which results when the internal carotid artery or its branches are damaged. If this latter occurs, the animal had best be killed before recovering consciousness, as it is impossible to control this haemorrhage. Damage to the brain can be avoided by previous intravenous injection of hypertonic saline; by use of a retractor well adapted to the surface of the brain; and by exercising patience and care when retracting and when swabbing the cerebrospinal fluid.

The post-operative complications consist of (i) inflammation and oedema of the conjunctiva of the eye on the operated side, (ii) gangrenous changes in the skin of the wound, (iii) infection of the wound, and (iv) interference with the masticatory mechanism. The conjunctivitis and oedema can be avoided or lessened by clipping the eyelids together before starting the operation. If this complication does occur it invariably heals if the eye is kept clean by bathing. The gangrene of the skin, as stated before, only occurs if the skin is dissected from the deeper fascial planes, but if the primary incision is clean and deep through skin and fascia and if the skin is kept moist throughout the operation, healing will occur with little difficulty. Infection of the head wound occurs easily unless rigid aseptic precautions are taken. The wound made for the insertion of the tracheal cannula was infected rarely. As the operation includes removal of one mandibular joint, mastication necessarily becomes asymmetrical, though only to a slight degree, owing to the fact that one-half to two-thirds of the ramus of the mandible and the corresponding internal pterygoid muscle are left intact. Normal chewing can, and indeed does occur, for the animal will masticate green food in a comparatively normal fashion within 24 hours of the operation. Milk should be supplied in the diet for the first 7 days. After several months the aperture in the skull becomes filled with partly calcified fibrous tissue and the pterygoid region filled with scar tissue. In one animal an attempted reformation of the jaw joint was noticed; a spicule of bone left in the wound had united with the ramus of the mandible and grown back to the skull just anterior to the bullat ympani, where a joint capsule had formed.

The advantage of this operation lies in the fact that *every stage of the operation is clearly visible to the operator*. The brain in the rabbit is small, the temporo-sphenoidal groove (middle fossa) is shallow, so that a good view can be obtained of the hypothalamic region. By this method also a greater variety of experiments can be performed than is possible by other methods. The hypothalamus may be stimulated electrically (for another method of performing this, see Harris, 1937), hypothalamic lesions may be made, various substances may be injected into the third ventricle, section of the hypophysial stalk and hypophysectomy may be performed. When performing hypophysectomy additional care is required to avoid haemorrhage from the cavernous sinus.

RESULTS

We have used this technique in two sets of experiments, first, for sectioning and making lesions in the pituitary stalk, and secondly, for compressing the stalk (without cutting it).

Lesions of varying extent were made in the stalks of twenty-six rabbits. The results of these operations may be grouped:

(i) Nine animals died during or immediately after the operation, the main cause of death being intracranial haemorrhage with resultant medullary anaemia.

(ii) Two rabbits lived for 3 days.

(iii) Seven rabbits lived between 5 and 50 days.

(iv) Whilst six rabbits lived for longer than 50 days, i.e. for periods of 55, 70, 92, 100, 125 and 218 days.

Seven of the animals in groups (ii) and (iii) appeared to have died from infection, as the conditions for asepsis were not good. It is, however, possible that their deaths were in part due to damage to the tuber cinereum, for the majority of these animals had convulsions prior to death. Bailey & Bremer (1921) noted in dogs that damage to the tuber often caused epileptiform convulsions.

As an example of our results, we quote here the case of rabbit 112, which lived for 70 days after the operation. From serial sections through the hypothalamus and pituitary, the stalk was seen to be entirely transected (Pl. I, fig. 1). The line of the lesion can be seen passing from one edge of the stalk to the other, the two cut ends having come into juxtaposition and united with fibrous tissue. This attempt at recovery is not functional however, for the nerve tracts and blood vessels in the stalk are interrupted by the scar tissue. In the sections it was found that on the pituitary side of the lesion, the stalk and posterior lobe were shrunken and very cellular (Pl. I, figs. 1 and 2). Also in some cases a large accumulation of colloid was observed in the posterior lobe. Crowe *et al.* (1910), Cushing & Goetsch (1910), noted similar effects. The cells of the anterior lobe appeared to have lost most of their cytoplasm and become rather atrophic.

In other experiments the stalk was compressed with a view to seeing on which side of the point of compression the blood would accumulate in the hypophysio-portal vessels. The operation was successfully performed in ten rabbits. In seven cases the stalk was pressed alone and in three cases the stalk and the internal carotid artery were taken together. Pl. I, figs. 3 and 4 show two such specimens—(the hypothalamus, stalk and pituitary gland having been fixed in situ and dissected out)—and it may be observed that the stalk is tense and swollen on the pituitary side of the point of compression. The detailed results of this operation will be included in a paper to be published by one of us (Gr. T. P.) in collaboration with Miss Una Fielding.

Fig. 2.

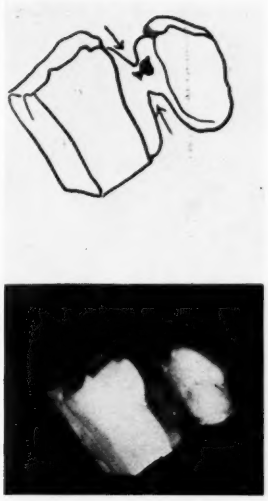


Fig. 4.

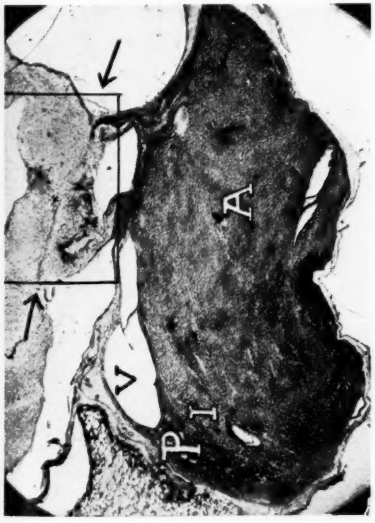


Fig. 1.

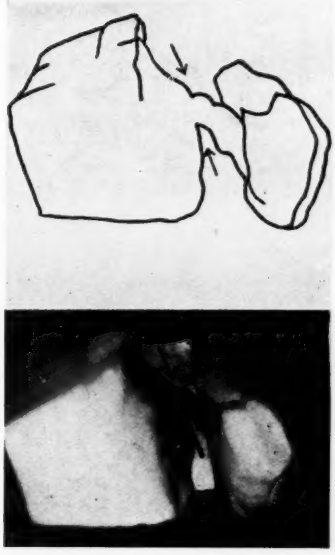


Fig. 3.

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SUMMARY

A description of a technique for operations on the hypothalamo-hypophysial region of the rabbit, with a criticism of the technique. Some experimental results, obtained by means of this operation, are briefly described.

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EXPLANATION OF PLATE I

Fig. 1. Microphotograph of a sagittal section through the pituitary gland, stalk and hypothalamus of rabbit 112, whose stalk was transected 70 days previously. Note, atrophic appearance of pituitary gland; line of lesion (indicated by two arrows), and vacuoles in posterior lobe and stalk (previously filled with colloid). (×16.) *A.* anterior lobe; *I.* intermediate lobe; *P.* posterior lobe; *V.* vacuoles (previously filled with colloid).

Fig. 2. Line of lesion (in Fig. 1) under higher power. Note fibrous tissue "healing" of the stalk and slight accumulation of basophilic cells on the pituitary side of the lesion, *B.* (×40.)

Fig. 3. Photograph and diagram of hypothalamus, stalk and pituitary gland, the stalk having been compressed immediately prior to death. Note the swelling of the stalk below the point of compression (indicated by arrows). (×5.)

Fig. 4. Photograph and diagram of a specimen similar to that in Fig. 3. Note again the enlargement of the stalk below the line of compression. (×3.)

(Figs. 1 and 2 are reproduced here by kind permission of the Royal Society from *Proc. roy. Soc.* B, vol. CXXII, p. 374, 1937.)

FURTHER REMARKS ON THE TERMINATIONS OF NERVES IN HUMAN TEETH

By O. W. TIEGS
Department of Zoology, University of Melbourne

In a recent paper on the subject of the innervation of the dentine, published in the *Journal of Anatomy*, Lewinsky & Stewart (1936) have criticized some earlier observations of my own (1932) relating to the same subject. As this criticism seems to rest on misunderstanding, I think it necessary to add a further note on the matter.

At the request of a dental colleague, who was much impressed with the sensitiveness of exposed dentine in his patients, I undertook, by means of the modern silver impregnation methods, to examine the dentine for stainable nerve fibres in its substance. This sensitiveness is stated to be particularly acute along the dentino-enamel junction, where the Tomes' fibres branch very freely. If, therefore, it is attributable to direct irritation of exposed nerve fibres, then we are justified in seeking such nerves even in the peripheral portion of the dentine.

Numerous human teeth, fixed in formalin and very slowly decalcified in hydrochloric acid were impregnated by the Bielschowsky block method and cut into sections 25μ in thickness. The usual high proportion of unsuccessful or only indifferent impregnations resulted. In one case however, a molar from a young adult, an extraordinarily brilliant impregnation was obtained and on this material the former and the present account is based.

The nerves, entering at the apical foramen, traverse the pulp in bundles which are usually associated with, and sometimes even ensheath, the blood vessels. Towards the periphery of the pulp they forsake the blood vessels, and break into smaller bundles from which isolated fibres arise that pass to the zone of the odontoblasts. They are particularly prevalent in the crown (Text-fig. 1). Where, and in what manner do they terminate? The answer to this question, as given in the previous paper, may be summarized as follows: (i) Numerous fine nerves, arising as branches from coarser fibres, pass through the zone of odontoblast cells and enter the "subdentinal zone" within which they turn and run, often with much branching, for considerable distances under the dentine (calcified dentine). They come into association with the Tomes' fibres by means of definite end-organs (cf. Figs. 2, 4 and 5 of my previous paper). (ii) But sometimes a branch from such a nerve will run along a Tomes' fibre as far as the orifice of a dentinal tube, i.e. into the inner margin of the calcified dentine itself (cf. Figs. 2, 4 and 5 of previous paper). The dentine along its inner margin becomes very black with Bielschowsky's method,

making accurate observation on the content of the tubes in that region very difficult (cf. especially Fig. 5 of previous paper). For this reason it is usually impossible to say with certainty how these nerves terminate. But immediately beyond the deeply staining zone, where precise observation is again possible, there is no longer a visible trace of these nerves, whence it seems likely that they have ended within the dentinal tubule along the inner margin of the dentine. (iii) Occasionally quite coarse fibres enter the "subdentinal zone"; and in some cases they may be observed to branch extensively amongst the Tomes' fibres, terminating on the latter by end-organs, while in other cases they run for considerable distances under the surface of the dentine without recognizable endings. (iv) Numerous exceedingly delicate nerve fibres may apparently never reach the "subdentinal zone" but seem to end, instead, on the bodies of the odontoblast cells (cf. Fig. 7 of previous paper). But even with the highest magnifications and with due attention to accurate illumination, it is impossible to be certain of this latter observation, for the shrunken surface of the large well-stained odontoblasts affords a very imperfect background on which to make precise observations as to a possible ending of these exceedingly delicate nerve filaments. Strong presumptive evidence in favour of such endings is, however, to be obtained from the fact that from the nerves which have passed through the odontoblast layer into the "subdentinal zone", filaments may turn back and re-enter the layer of odontoblast cells.

These observations appear then to show that the relationship of the nerves is with the odontoblasts and their processes (Tomes' fibres) and not with the dentine.

After the completion of this work Sealey began observations on material prepared by the same method and also succeeded in tracing occasional nerve fibres as far as the orifices of the dentinal tubes, but not along the tubes (Sealey, 1932). His observation in this respect does not, then, differ from my own.

Despite this obvious agreement Lewinsky & Stewart then reinvestigated the problem because "the discrepancies in the results of these two investigators appeared to justify further work being undertaken on the problem", and by means of preparations which do not even trace the nerve fibres into the calcified dentine, but only into the odontogenic zone, conclude in favour of Sealey.

Two factors in my presentation of the subject appear to have caused the misunderstanding:

(i) The fact that I "unfortunately only published drawings" of my preparations. Yet photographs can give but an inadequate and very fragmentary picture of the path of the delicate nerve fibres with which we are concerned, for at the high magnifications which it is necessary to employ, the divergence of a nerve fibre by more than $0{\cdot}5\mu$ (depth of focus of 2 mm. immersion lens) out of the plane of focus renders it invisible. Nevertheless as some form of objective evidence is needed, several photographs, which have been taken for me by Dr E. S. J. King of the Melbourne University Pathology Department,

are given in the present paper. Two of these have been selected because they refer to objects actually illustrated in the previous paper.

(ii) The use of the term "subdentinal zone". By this I mean the very narrow zone between the layer of odontoblasts and the inner margin of the calcified dentine. It is known by a variety of other names: odontogenic zone, dentogenetic zone, dentinoid zone, predentin, uncalcified dentine.

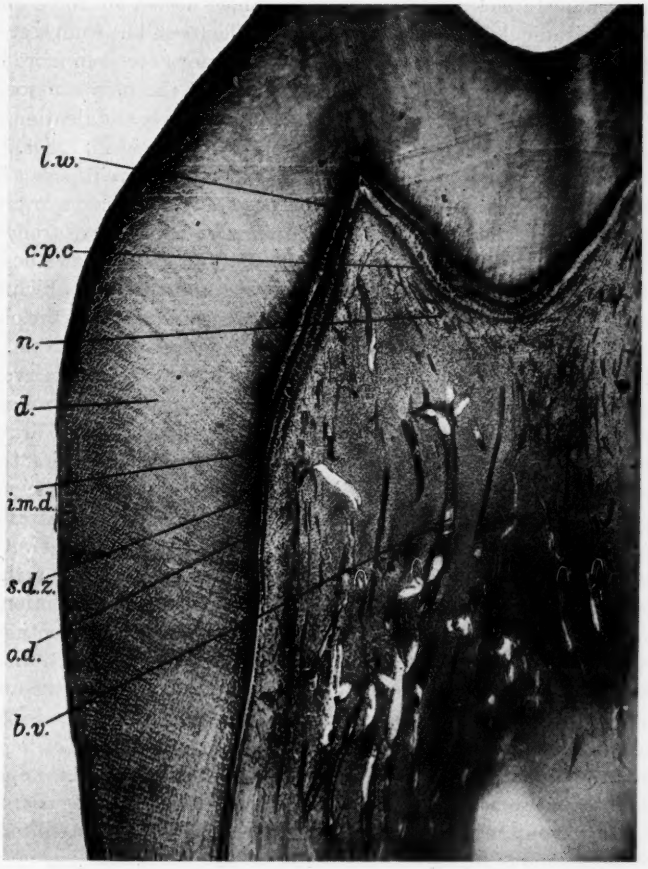
In my material it appears, in the cervical region of the tooth, as a clear structureless zone traversed by the Tomes' fibres, the outer portion, adjacent to the dentine, staining pale brown with the Bielschowsky process, this stainable portion merging into an almost, or even completely unstained part nearer the odontoblasts (cf. Pl. II, figs. 4, 5, 7, 8 and 9; Pl. III, figs. 13 and 16). Towards the crown of the tooth the more deeply staining part gradually encroaches upon the other, and may even completely replace it. It is here organized into well-defined dentinal tubes and is evidently dentinal matrix awaiting calcification (Pl. III, figs. 15 and 17; Pl. IV, figs. 18, 19, 20 and 21). That part of the subdentinal zone in which there is visible evidence of dentine formation I have referred to as "odontogenic"; where this is not seen I have employed the non-commital term "subdentinal zone".

For present purposes it is not necessary to decide whether this zone, particularly when there is no visible indication of dentinogenesis, is to be reckoned as part of the dentine[1] (thereby justifying the use of the term "innervation of dentine" from the occurrence of nerves in it), for nerves may sometimes actually be seen running right through it into the inner margin of the true dentine. The question at stake is not whether nerves ever enter the calcified dentine, for that they undoubtedly do, but whether they run through the dentine even into the outer portion, where it is said to be exceptionally sensitive.

Text-fig. 1 will prevent any further ambiguity. This is a photograph of one of the sections on which the present work is based. The outer thick zone (*d*) is the dentine, the enamel having disappeared during decalcification. The inner margin (*i.m.d.*) of the dentine, it will be observed, stains very black, rendering observations on any nerves contained therein very difficult. Below this is the narrow subdentinal zone (*s.d.z.*), seen at higher magnification in Pl. II, figs. 5, 7 and 9. Then follows the layer of odontoblasts (*o.d.*), under which is the layer of Weil (*l.w.*), best seen in the crown of the tooth, and composed of numerous delicate fibrils which are partly derived from the odontoblasts

[1] Although it is in this zone that dentine formation occurs, yet this zone does not disappear in the completed tooth, but is seen even in teeth of elderly persons. It seems, therefore, that with the progress of dentinogenesis within it, along the inner margin of the dentine, the zone recedes towards the pulp. If it were entirely dentinal matrix awaiting calcification, then any nerves contained within it would be enclosed in the calcified matrix and probably destroyed; but that this does not generally occur is shown by examining places where dentine formation is still in progress, e.g. Pl. IV, fig. 20. The forming dentine here seems to push the nerves before it rather than enclose them, and for that reason it seems very doubtful whether the nerve-containing part is dentinal matrix. Whether this interpretation is right or wrong, it is obvious that the observations reported in this paper are not affected by it.

and partly from a zone of condensed pulp cells just below it (*c.p.c.*). The layer of Weil is often regarded as a shrinkage artefact; yet in Bielschowsky preparations its fibrillar texture can be beautifully demonstrated, and these fibrils certainly show no indication of stretching (cf. Pl. IV, fig. 18).



Text-fig. 1. Part of longitudinal section of the tooth, on which the present account is based. Bielschowsky preparation. *b.v.* blood vessel of pulp; *c.p.c.* layer of condensed pulp cells; *d.* dentine; *i.m.d.* inner margin of dentine, very deeply stained; *l.w.* layer of Weil; *n.* nerves, no longer associated with blood vessels, but separating into single fibres; *od.* layer of odontoblasts; *s.d.z.* subdentinal zone.

With these preliminary remarks we may now proceed to substantiate the observations reported in the previous paper.

In my material the subdentinal zone in the cervical region of the tooth

appears clear and quite structureless and forms an ideal background for the examination of the finer nerves contained within it. From the periphery of the pulp rather thin but deeply staining nerves pass outwards through the zone of odontoblasts into the subdentinal zone. Here they usually turn and run, often with much branching, parallel with the inner surface of the dentine. The most complete pictures of the course of these nerves are to be obtained, not from longitudinal or transverse sections of the tooth but from sections cut tangentially along its margin; semi-tangential sections are even more instructive, since they exhibit not only the branching of the nerves amongst the Tomes' fibres, but also their relation to the odontoblasts and dentine.

Drawings from two sections cut in this way are shown in Pl. I, figs. 1 and 2; they are taken from the cervical region rather high up where it passes into the crown of the tooth. Above we see the deeply stained inferior margin of the dentine, with the tubules cut not longitudinally but almost transversely. Below is indicated the zone of the odontoblasts. Between the two lies a wide strip of obliquely cut subdentinal zone, in the outer part of which tubules are already becoming distinguishable. The zone is traversed by the obliquely cut Tomes' fibres on their way from the odontoblasts to the dentine.

In Pl. I, fig. 1 we see to the right three thin but deeply stained nerves which have passed through the odontoblast zone into the subdentinal zone, within which each then proceeds to branch. The whole has been carefully drawn with the camera lucida, the path of the fibres throughout the entire thickness (25μ) of the section being projected on to the single plane of the drawing; the apparently free terminations of the nerves represent in most cases severed nerves only, for they occur at the surface of the microtome section. Of these three nerves the one to the left is particularly noteworthy for the great expanse of its branching; as the accompanying scale of magnification shows, its branches can be followed for as much as 0·2 mm. under the dentine. Compared with the main fibres from which they have arisen, these fine fibrils always stain rather weakly, while the finest—distinguishable always by the numerous minute varicosities which they exhibit—usually appear very pale, and can be seen only with the highest magnification. It is doubtless owing to this refractoriness to our staining methods that we so seldom obtain preparations showing these fine branching fibres in the subdentinal zone. The coarse fibres, on the contrary, appear to stain without much trouble, for we often obtain them in preparations which reveal nothing of the finer branches.

Pl. I, fig. 2 will serve as an additional illustration of this type of nerve fibre. The nerve enters the subdentinal zone at the extreme right lower corner of the drawing, and branches widely within it. Numerous other delicate fibres also are present, but their source of origin lies outside the thickness of the section.

Objective evidence, by means of photographs, for the existence of this system of fine branching fibres is difficult to present, for the reason already stated. In Pl. II, fig. 3, however, is shown a case where sufficient of this system

could be obtained precisely within the plane of focus to make an intelligible photograph possible. The photograph reproduces the nerves drawn in the middle of Pl. I, fig. 2; its position will be recognizable by the thin fibre bending sharply upon itself, while several other adjacent fibres are also distinguishable.

It will be evident from the illustrations given by Lewinsky & Stewart that they can have seen nothing of this system of delicate branches; for although magnifications are not given, it would seem that they had before them some of those exceptionally coarse fibres—of a thickness at least equal to the Tomes' fibres—such as one occasionally encounters in the subdentinal zone (cf. Pl. IV, figs. 18 and 19).

Despite the successful staining of the most delicate nerve fibres which the present preparations show, I have not found any indication of a closed nerve-net at the periphery of the pulp, as described by Riegele (1934) and Berkelbach van der Sprenkel (1935). Were it of general occurrence we would certainly expect some indication of it in a preparation such as that drawn in Pl. I, figs. 1 and 2.

We now turn to the question of the possibility of nerves entering the dentine from the subdentinal zone, and passing along the dentinal canals to the periphery of the dentine.

Despite the excellence of the staining in the subdentinal zone there is no trace of visible nerves in any part of the dentine. In the matter of establishing a negative conclusion we can, of course, only operate within the limits of our method; yet we are not, in this case, so completely the victims of a capricious technique as some writers urge. With so excellent a staining of the most delicate nerves just under the dentine we would expect to find at least some staining of nerves within its substance, were these present as extensively as has been claimed. It is possible that some factor in the dentine is inhibiting their staining. But in that case we would at least expect to find a general tendency of the delicate fibrils of the subdentinal zone to place themselves alongside the Tomes' fibres and pass with them to the dentine; this might, for example, be expected in the sections drawn in Pl. I, figs. 1 and 2 where fibrils sometimes just perceptible with the highest magnifications have been successfully impregnated. Yet of the hundreds of Tomes' fibres shown in these drawings there is not a single case of such an association. Nevertheless in working through the preparations cases are sometimes encountered where such an association does indeed occur; these exceptional cases are discussed further below.

Reference should be made here to a peculiar form of artefact which is apt to prove very deceptive and give the false impression of nerves accompanying the Tomes' fibres into the dentine. This arises from the fact that the Tomes' fibres often exhibit much surface distortion, the ridges so produced staining deep black with Bielschowsky's stain, and frequently showing a marked resemblance to well-stained nerves. An excellent example of this is seen in the photograph in Pl. III, fig. 14; while the surface distortion on the Tomes'

fibre to the left of the picture is too obvious to mislead, the fibre to the right appears to have a well-stained nerve accompanying it. Yet on close examination this proves not to be a nerve at all, for it is not continuous with any nerve in the pulp, but arises as a ridge from the odontoblast cell. When distortions on the Tomes' fibres may so closely simulate nerves, what reliance can be placed on statements in the literature that nerves have been detected accompanying the Tomes' fibres into the outer portion of the dentine, when it is quite impossible to trace those apparent nerves back to unquestionable nerves in the pulp?

The foregoing observations seem to suggest, then, that direct stimulation of nerves in the periphery of the dentine is not the cause of its sensitiveness. If the Tomes' fibres transmit some forms of stimulus to the nerves, as Tomes, Walkhoff, Hopewell-Smith and others suggest, then a connexion of these nerves with Tomes' fibres and odontoblasts is to be expected. That such connexions do indeed occur, even in large numbers, can readily be seen in the preparations, the nerves forming distinct end-organs, not unlike the minute "end-feet" (boutons terminaux) with which we are familiar in the central nervous system.

There is much variation in their form. One of the best defined types, though uncommon, is an elongate leaf-like form of ending which becomes applied to the surface of the Tomes' fibre. An example is shown at e_1 in Pl. II, fig. 4, this particular nerve having already been illustrated in the previous paper (cf. Fig. 2 of that paper). The drawing has been carefully made with the camera lucida. The nerve is seen on the right penetrating the odontoblast layer and entering the subdentinal zone. Here it turns, branches, and becomes associated with two of the Tomes' fibres by distinct end-organs, of which one is seen in face view, the other (e_2) in profile. The example has been selected because sufficient of the nerve occurs in the plane of focus to make a photograph possible. In this photograph (Pl. II, fig. 5) one of the end-organs (e_1) is clearly seen; the other, being out of focus, is recognizable only as a faint blur. A drawing of the ending visible in face view is shown at still higher magnification in Pl. II, fig. 6.

Usually the endings are more rounded or conical. A good example, seen in face-view is given in Pl. II, fig. 7. Such endings may occur either at the termination of a fibre (Pl. II, fig. 7) or, as in Pl. II, fig. 8, along the course of a fibre. The nerve shown in the last drawing exhibits several end-organs, of which one has been reproduced photographically in Pl. II, fig. 9. Pl. III, fig. 10 is a photograph of another ending, seen this time in profile, the Tomes' fibre being cut obliquely; the nerve here shown is a fragment of the larger branching nerve drawn in Fig. 6 of the previous paper, and will be recognizable in the right bottom corner of that drawing. Other similar endings are shown in Pl. III, figs. 12 and 16, of the present paper.

If now we turn to Pl. I, figs. 1 and 2, numerous examples of such end-organs associated with the Tomes' fibres will be encountered; they are especially well seen in Pl. I, fig. 2 and several are also recognizable in the photograph

(Pl. II, fig. 3), which is a fragment from Pl. I, fig. 2. One of the endings (e_1) is particularly well seen in this photograph; it is clearly not a varicosity on the nerve, but is an outgrowth from it, and is, moreover, closely applied to the surface of the Tomes' fibre. The resemblance of these endings to the "boutons terminaux" of the central nervous system is unmistakable.

It is not possible everywhere to identify endings with such assurance, and particularly is this the case for the very finest nerves, where the fibres are of such delicacy that they are recognizable only as a row of excessively minute varicosities, just visible with the highest magnification, and with the connecting thread between the varicosities beyond the power of the microscope to reveal. This may seem an exaggeration; yet actually they are not uncommon in the preparations. A fibre approaching this degree of delicacy is seen at x in the photograph Pl. II, fig. 3; others, rather finer, appear in the drawing Pl. III, fig. 11. The very finest it is scarcely possible to illustrate. It is hardly to be expected that fibres of this extreme delicacy should develop easily recognizable end-organs, and it is not unlikely that some of the minute "beads", in cases where they lie against the Tomes' fibre, may not be varicosities at all, but points of contact with the Tomes' fibres. The example shown in Pl. III, fig. 11 has been chosen for illustration because some of the very delicate nerves there present exhibit identifiable endings; but if such minute endings occurred along the course of a nerve, and not at its termination, it would be impossible to identify them with any certainty as true end-organs.

Apparent exceptions to the general rule that the nerves end on the Tomes' fibres and avoid the dentine are sometimes encountered. Here a nerve fibre will be observed becoming associated with a Tomes' fibre and passing with it to the orifice of a dentinal tubule, i.e. to the inner margin of the dentine itself. Usually such a fibre arises as a branch from one of the fine nerves that run along the under surface of the dentine (cf. y in Pl. II, figs. 4 and 8). But sometimes fibres are encountered that proceed direct to a dentinal tube. A particularly good example of this is seen in Pl. III, fig. 13, where a surprisingly coarse and deeply staining nerve passes straight through the subdentinal zone and divides just below the dentine into two, each branch going to the orifice of a dentinal tube.

In what manner do these nerves end? This question cannot be answered with certainty, for the inner margin of the dentine stains very black with the Bielschowsky method, usually completely obscuring the nerve as it enters the dentinal tubule. Yet immediately within the dentine precise observation is again possible, and then the nerve is no longer to be seen. If the nerve in the subdentinal zone stains as deeply as the one shown in Pl. III, fig. 13, we could scarcely fail to detect it within the dentinal tube, if it actually passed along it; yet the preparation shows no indication of it. It seems most likely therefore that the nerve has ended on the Tomes' fibre at the opening of the dentinal tube, the ending being obscured by the very deep staining of the dentine. In fortunate cases endings may actually be seen in this position (cf. Fig. 4 of my

previous paper); Pl. III, fig. 17 shows an ending immediately below the dentinal orifice.

In Pl. III, fig. 16 is shown an unusual case of a nerve passing through the subdentinal zone to the dentine but independent of a Tomes' fibre. It is a deeply staining nerve, with delicate side branches, the main fibre ending short at the inner surface of the dentine, but not at the orifice of a dentinal tube. The most likely explanation of such a case is that the main fibre has been caught up in the process of calcification, the part included in the dentine having been thereby destroyed.

The foregoing observations relate particularly to the cervical region of the tooth, this having been selected for minute description because, in my material, the background of the nerves is not obscured by marked dentinogenesis in the subdentinal zone. In the crown, however, dentine formation does not appear to have ceased, and a well-defined uncalcified matrix is present (Pl. III, figs. 15 and 17; Pl. IV, figs. 18, 19, 20 and 21). As in the cervical region of the tooth we can again distinguish the delicate fibres running under the surface of the dentine and coming into relation with the Tomes' fibres by means of small end-organs (Pl. IV, fig. 21); most of them, however, I have not succeeded in following for such long distances as in the cervical region, but am unable to decide whether this is due to the difficulties of observation, or to defective staining, or to the paucity of such very long filaments. In addition to these nerves there occur in the crown, in my material, an unusually large proportion of delicate nerves which, while forming end-organs on the Tomes' fibres, undergo scarcely any branching at all (Pl. IV, fig. 21).

There occur also in the crown a small number of extremely coarse nerve fibres—sufficiently coarse to be seen with a $\frac{2}{3}$ in. objective. These usually undergo remarkably little branching in the subdentinal zone, but run as surprisingly coarse fibres sometimes for considerable distances under the dentine. The fibre shown in Pl. IV, fig. 20 is particularly remarkable; as the accompanying scale of magnification shows, it can be followed for 0·2 mm. before it gives rise to the thin weakly staining branch (*b*) at the left of the drawing. Other examples are shown in Pl. IV, figs. 18 and 19; fig. 18 is an untouched photograph and shows some of the nerves in the subdentinal zone; fig. 19 is the same photograph, on to which there has been sketched the complete course of these nerves as far as this occurs in this particular section. Fibres of this kind occur, in my material, only in the crown of the tooth. End-organ formation in these nerves is confined to the finer branches which arise from them.

It is worth noting that in the crown, where there is a well-defined zone of odontogenesis within the subdentinal zone, the nerves that run parallel to the undersurface of the dentine lie actually beneath the zone of odontogenesis, and not within it. This is well seen in Pl. IV, fig. 20, and is also recognizable in the photographs, Pl. IV, figs. 18 and 19. It seems that as odontogenesis proceeds from the inner margin of the dentine in the direction of the pulp it forces these nerves before it; if this did not occur the nerves would become

caught up during calcification and probably destroyed. But in cases where the nerves run along the Tomes' fibres they may be traced well into the zone of odontogenesis (Pl. III, fig. 15; Pl. IV, fig. 21); and if this zone does not stain too deeply may be observed forming apparent end-organs on the Tomes' fibres, even as far as the orifices of the dentinal tubes (Pl. III, fig. 17).

In addition to the nerves already described there occurs in very large numbers, mainly in the crown of the tooth, another class of nerve fibre, which seems to end on the bodies of the odontoblast cells, without ever entering the subdentinal zone. Just below the layer of Weil the deeply staining nerves proceed to branch considerably. Some of these branches pass as thin well-stained fibres into the subdentinal zone, forming end-organs there; but many, indeed the majority, are exceedingly thin and weakly staining, and distinguishable often with difficulty from the fibrillae which comprise Weil's layer. As these nerves occur in great numbers one might have expected little trouble in determining their mode of ending; yet in practice this has proved a most difficult matter, for the odontoblasts stain so much more deeply than these delicate nerves, that it is usually impossible to follow the latter clearly, once they have reached the level of the odontoblast cells. Despite this, however, I have not infrequently been able to observe nerves definitely ending at the surface of the odontoblast cells (cf. Fig. 7 of my previous paper); but in no case has a distinctive end-organ ever been recognizable in association with them, so that it is not possible to decide whether they are actual endings of nerves or merely the result of an interruption in their staining. It seems probable, however, that the former will prove to be the case, for quite commonly, both in the crown and in the neck of the tooth, nerves may be seen turning back from the subdentinal zone and re-entering the zone of odontoblast cells. Examples will be seen in Pl. I, figs. 1 and 2 and at *x* in Pl. II, fig. 4, the nerve shown in the latter figure being also distinguishable for part of its course in the photograph Pl. II, fig. 5. Pl. II, fig. 4, incidentally, will serve to show the uncertainty of identifying a true ending for such a nerve.

As to the manner of functioning of the nerves of teeth we can, of course, in the absence of experimental data, only speculate. It is evident that the anatomical arrangement is such as might be expected on Tomes' theory that it is the odontoblast processes (Tomes' fibres) that transmit some form of stimulus to the nerves of the pulp. While there is no direct evidence for this theory, it would seem that the objections which are commonly brought against it are unconvincing. Thus, the untenability of Tomes' view has been urged on the ground that "the odontoblast cells are mesodermic in origin and it is generally accepted that mesodermal tissues do not conduct nerve impulses" (Stewart, 1936). Yet it is not suggested that the Tomes' fibres are nerves, but only that they may transmit some form of stimulus to the nerves of the pulp. Stimulus transmission is not an exclusive property of ectodermal tissues—skeletal muscle fibres, for example, are able to transmit stimuli with a remarkable resemblance to nerve impulses. In any case so many exceptions have been

found to the doctrine of germ-layer specificity, that it is impossible at the present day to raise it as a valid criticism against Tomes' views; in the case of teeth, for instance, it is known that in some fishes the enamel arises not from ectoderm but from endoderm (Graham Kerr, 1919).

Uncertainty also attaches to the physiological significance of this surprisingly rich supply of nerves, and to the meaning of the sensitiveness of the dentine.

It is a matter of common experience that the localizable tactile sensations from a tooth survive the destruction of its pulp, and some preliminary measurements by Stewart (1927) seem to show that it is even quantitatively unaffected. These sensations have their origin evidently in the periodontal membrane and not in the tooth itself, despite its surprisingly rich nerve supply. That the nerves may exert some "trophic" function, possibly stimulating the formation of secondary dentine as the outer hard layers of the tooth become worn away, may in the future require consideration; observations on secondary dentine formation in teeth with degenerated mandibular nerves would solve the question.

Meanwhile the only known response to irritation of the dentine is a sensation of pain and this suggests that the function of the rich nerve supply of the pulp is to be sought along other lines. Of all the organs in the body the teeth are the most subject to mechanical injury by contact with hard foreign bodies. It would be surprising therefore if some mechanism did not exist to protect them from undue strain. The momentary sharp pain that is felt within a tooth when the force of the bite is concentrated on it by unexpectedly encountering a piece of grit in the food, is a familiar experience. Local deformation of the dentine in this case calls forth a sensation of pain. May it not be the function of the intrinsic nerves of the tooth to prevent, reflexly, the application of stresses which it is beyond the power of the tooth to withstand?

SUMMARY

In a decalcified molar from a young human adult, stained by Bielschowsky's method, nerves have been seen in great numbers passing through the layer of odontoblast cells, and entering the very narrow zone (subdentinal zone) between these and the calcified dentine. Here most of them run amongst the Tomes' fibres as very delicate and often highly branched fibrils. Sometimes however, particularly in the crown of the tooth, fibres may fail to branch, and remain in association with the Tomes' fibres. In the crown, but not elsewhere, exceptionally coarse fibres have also been observed in the subdentinal zone.

The nerves end on the Tomes' fibres by distinct end-organs, not unlike the "boutons terminaux" of the central nervous system.

Sometimes a fibre may pass right through the subdentinal zone to the orifice of a dentinal tube, but such nerves have never been observed passing

along the tubes; no evidence has therefore been obtained for the occurrence of nerves in the peripheral part of the dentine, which is said to be exceptionally sensitive.

The anatomical arrangement is such as would be expected from Tomes' hypothesis that the fibres which bear his name conduct stimuli from the dentine to the nerves of the pulp.

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EXPLANATION OF PLATES I–IV

Plate I

Figs. 1 and 2. Two drawings of the subdentinal zone (*S.D.Z.*) from the cervical region of the tooth high up near the crown. The section has been cut very obliquely so that, instead of a single row of Tomes' fibres passing direct from the odontoblast cells to the dentine we see numerous rows of fibres, obliquely or even transversely cut. At the top of each drawing the inner margin of the dentine (calcified dentine) is shown (*D*), with the dentinal tube cut almost transversely; at the bottom of each drawing the odontoblast layer (*OD*) is just indicated. All the nerves present in the 25μ-thick section have been drawn by the camera lucida on to the plane of the drawing. Numerous end-organs recognizable, especially in fig. 2.

Plate II

Fig. 3. Photograph of portion of the section drawn in fig. 2, and taken at about the same magnification. The photograph corresponds to the middle portion of fig. 2, and its position will readily be identified by the thin nerve fibre which bends sharply upon itself. The photograph has been taken to bring an end-organ (e_1) on this nerve into sharp focus; two other endings e_2 and e_3 are also seen. At x is shown one of the nerves approaching in delicacy the very fine nerves referred to in the text.

Fig. 4. Fragment from the cervical region of the tooth, showing on the right of the drawing a deeply staining nerve passing through the layer of odontoblast cells and entering the subdentinal zone where it branches, the main branch turning to the left and travelling under the surface of the dentine. From this branch a nerve (y) is seen going off to the dentine, while another (x) turns downwards and re-enters the odontoblast layer. The latter nerve illustrates well the difficulty of coming to any decision as to the manner of ending of nerves among the odontoblast cells; in this case it is uncertain whether there is a minute terminal end-organ or whether it is merely a varicosity at which the staining has been interrupted. *D.* dentine; *OD.* odontoblasts; *S.D.Z.* subdentinal zone.

Fig. 5. Photograph of portion of the same with one of the end-organs (e_1) exactly in focus; the adjacent end-organ is not in the plane of focus and is seen only as a blur. Much of the nerve itself is sufficiently in the plane of focus to appear in the photograph; note especially the branch x returning towards the zone of the odontoblasts.

Fig. 6. Drawing at higher magnification of the end-organ (e_1) of the previous figure.

Fig. 7. Photograph of a fragment from the cervical region, showing a thin nerve forming a conspicuous end-organ (e) on a fibre of Tomes. *D.* dentine; *OD.* odontoblasts; *S.D.Z.* subdentinal zone.

Fig. 8. Drawing of a fragment from the cervical region, showing a nerve branching in the subdentinal zone (*S.D.Z.*), with three end-organs attached to the Tomes' fibres. Note also that a branch from this nerve (*y*) passes with a Tomes' fibre to the inner margin of the dentine (*D*).

Fig. 9. Photograph of portion of the same with one end-organ (*e*) sharply in focus. This ending will be recognized on the left in Fig. 8.

PLATE III

Fig. 10. Photograph, at higher magnification, of portion of the branching nerve which has been drawn in Fig. 6 of the previous paper (the nerve will be recognized near the right bottom corner of that drawing). Note that one of the branches from the nerve forms a conical end-organ (*e*) on a Tomes' fibre. The section is from an almost tangentially cut part of the subdentinal zone.

Fig. 11. Drawing of an obliquely cut fragment of the subdentinal zone, showing a very delicate nerve branching, among the Tomes' fibres, into yet finer filaments, some of which form end-organs (*e*) on the Tomes' fibres.

Fig. 12. Drawing of a small fragment from the cervical region, showing a thin nerve with four end-organs on the Tomes' fibres in the subdentinal zone. *D.* dentine.

Fig. 13. Drawing of a fragment from the cervical region showing a deeply stained coarse nerve, passing through the subdentinal zone (*S.D.Z.*), and branching just below the dentine (*D.*) into two parts, each of which passes to the opening of a dentinal tube.

Fig. 14. Photograph of a fragment from cervical region, showing surface deformation of the Tomes' fibres. One of these, indicated by *x* shows a remarkable resemblance to a nerve accompanying a Tomes' fibre. *D.* dentine; *OD.* odontoblast.

Fig. 15. Drawing of fragment from crown; note in the subdentinal zone a well-developed region of odontogenesis (*ODG.*), in which calcification is beginning. Three conspicuous nerves pass, each in association with a Tomes' fibre, into the zone of odontogenesis. Any end-organs which may be present are obscured by the depth of staining of the odontogenic zone. From one nerve a small side branch arises.

Fig. 16. Drawing of fragment from the cervical region, showing a deeply stained nerve fibre passing through the subdentinal zone to the inner margin of the dentine where it stops short. It is not associated with the orifice of any dentinal tube.

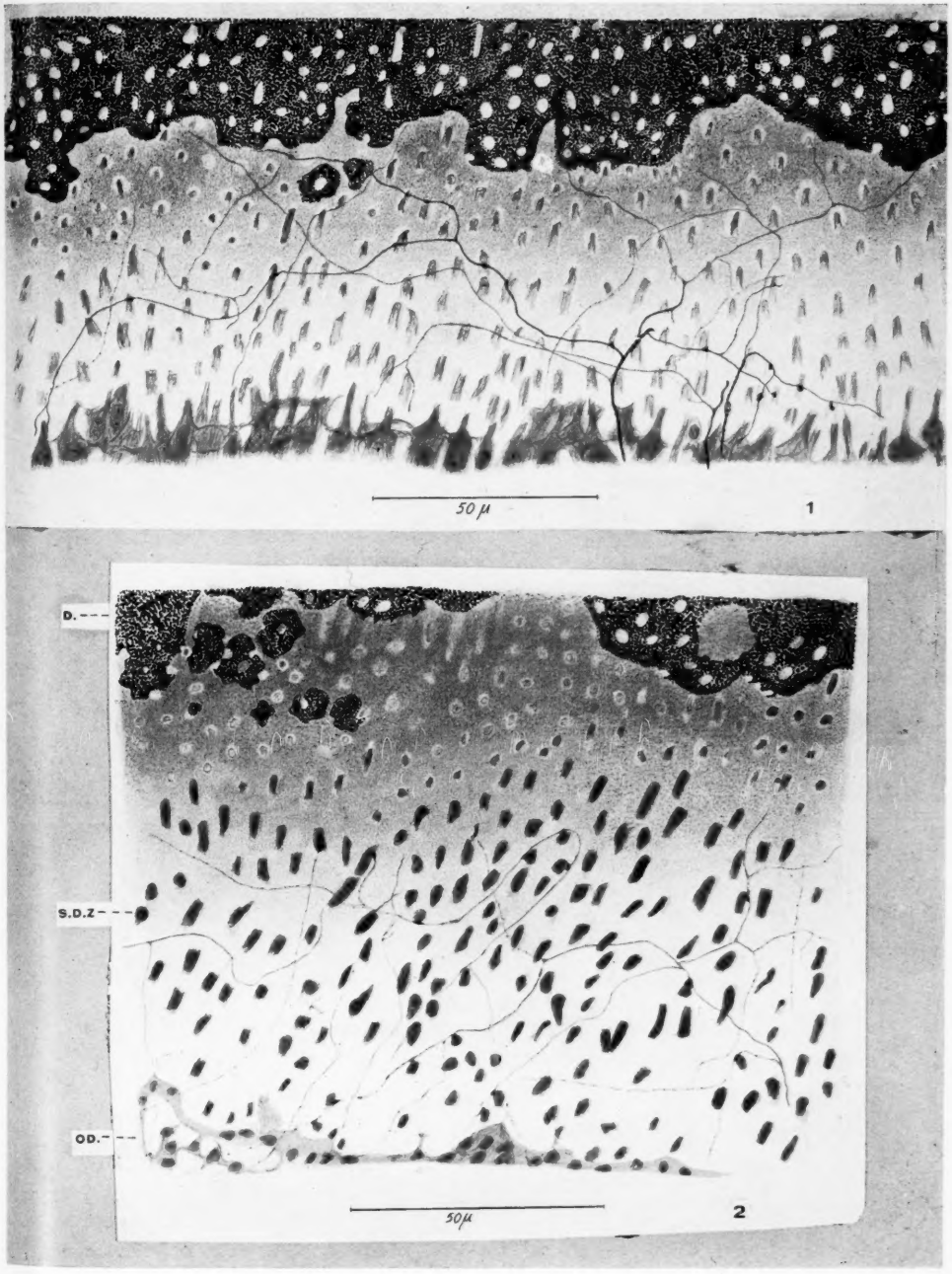
Fig. 17. Drawing of a fragment from the crown, showing a well defined zone of odontogenesis (*ODG.*) in the subdentinal zone. Portion of a branching nerve is shown with end-organs on the Tomes' fibres, one of these just below the orifice of a dentinal tube. *D.* dentine; *OD.* odontoblasts.

PLATE IV

Figs. 18 and 19. Two photographs from the crown of the tooth, showing three of the exceptionally coarse nerves which occur here. Fig. 18 is an untouched photograph; fig. 19 is the same photograph, into which the complete course of the nerves throughout the thickness of the section has been sketched. Note the well-defined zone of odontogenesis (*ODG.*) in the subdentinal zone. The dentine (*D.*) stains very deeply along its inner margins. Note the well-developed layer of Weil (*l.W.*).

Fig. 20. Drawing of a similar region from the crown, to show one of the exceptionally coarse nerve fibres (*N.*) in the subdentinal zone, just below a well-defined region of odontogenesis (*ODG.*). *D.* dentine; *OD.* odontoblasts.

Fig. 21. Drawing from the crown of the tooth, the subdentinal zone exhibiting a well-defined zone of odontogenesis (*ODG.*), within which calcification is beginning. Seven very delicate nerves are present in the section, forming end-organs on the Tomes' fibres, sometimes even within the zone of odontogenesis. *D.* dentine; *OD.* odontoblasts.



TIEGS—Further Remarks on the Terminations of Nerves in Human Teeth

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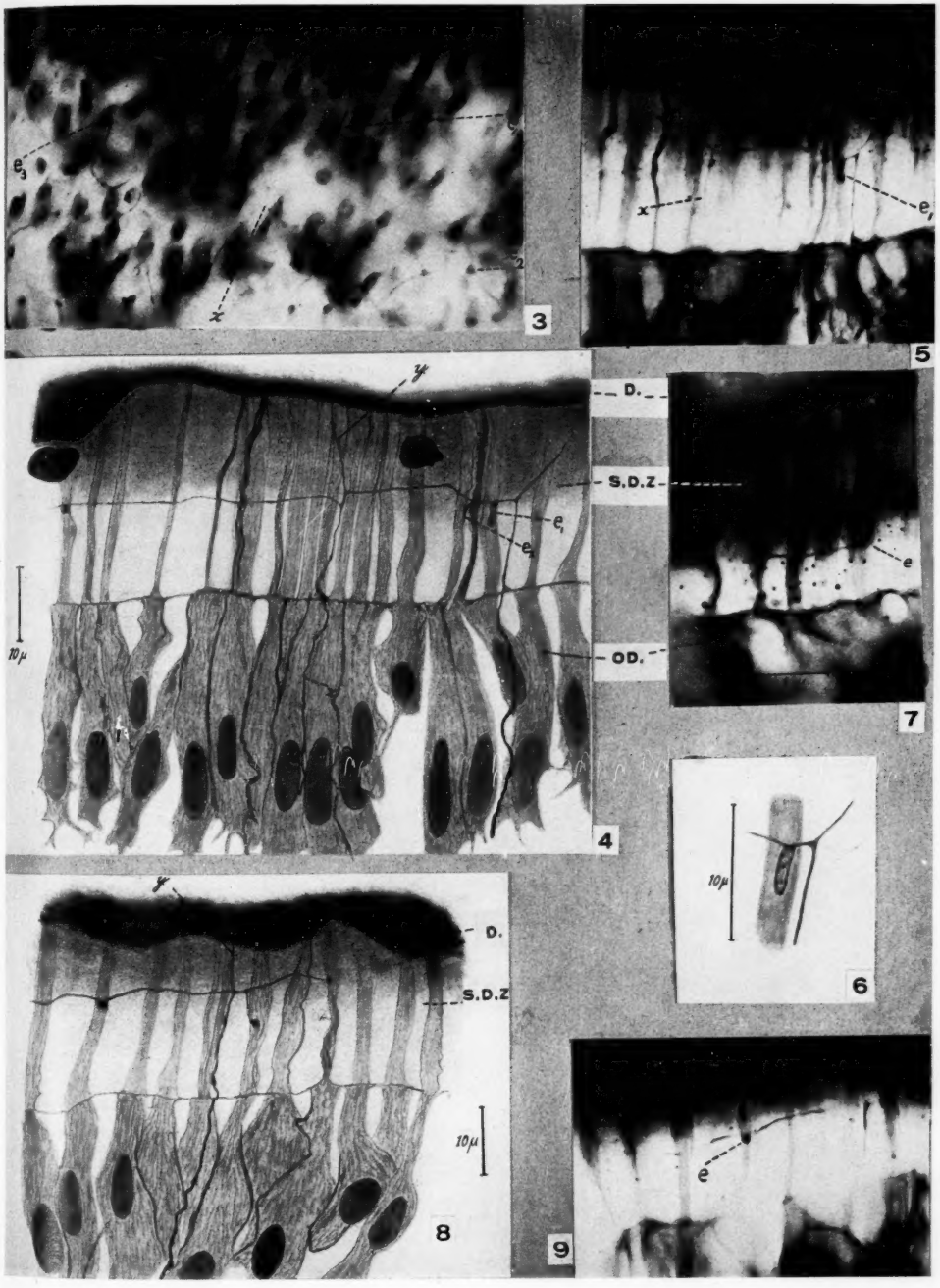

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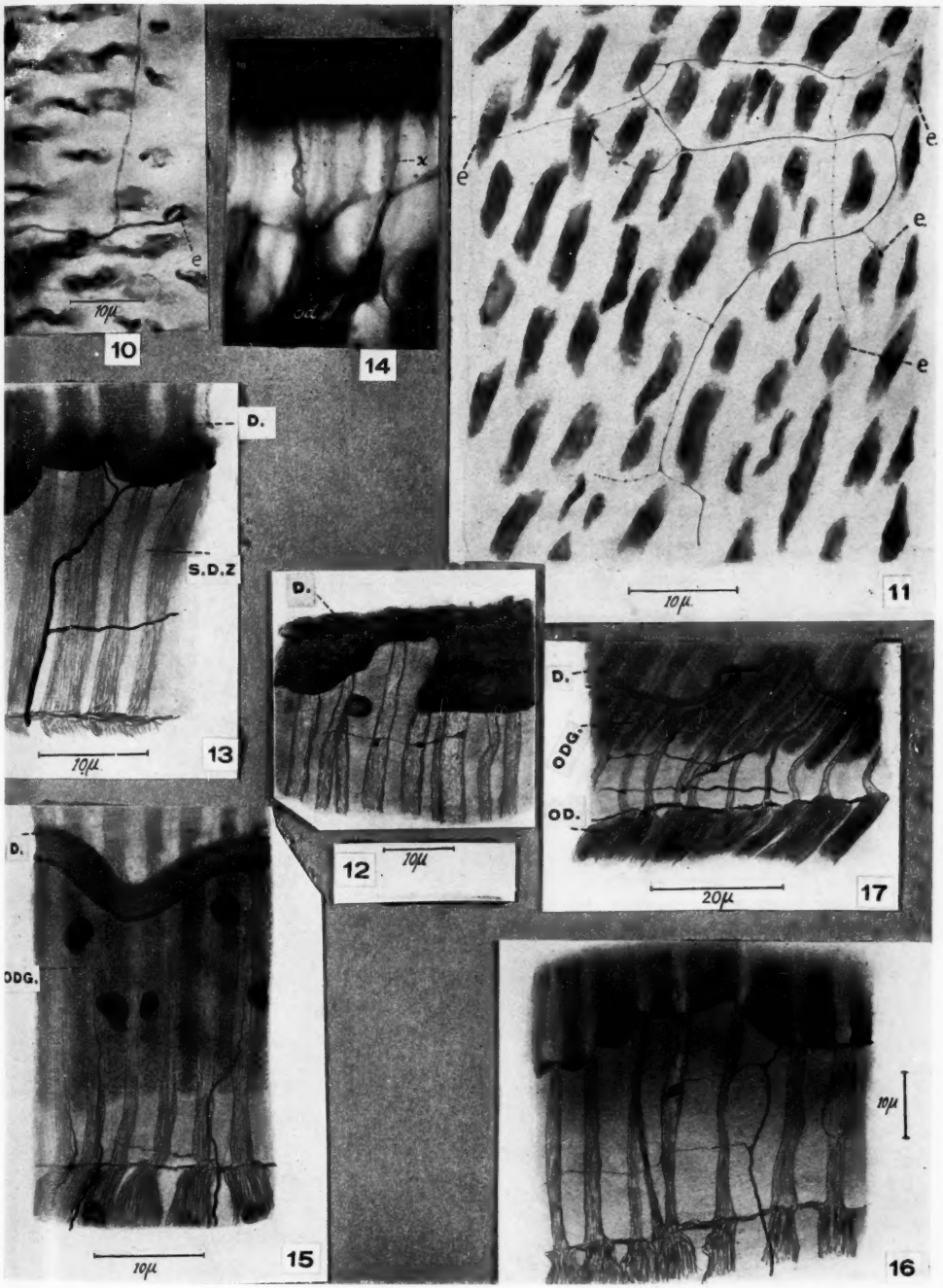

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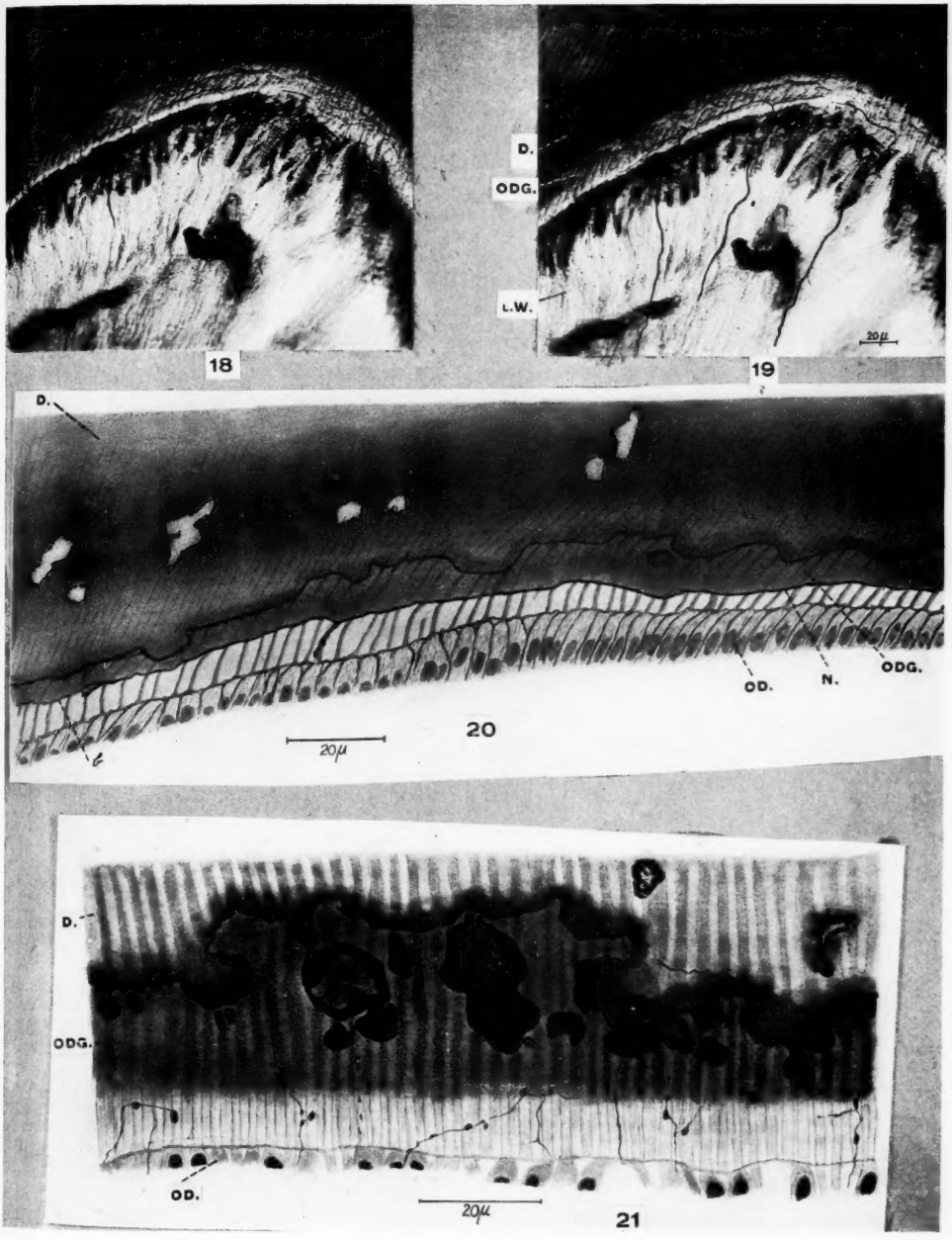

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THE DEVELOPMENT OF THE PARS MEMBRANACEA SEPTI IN THE HUMAN HEART

By P. N. B. ODGERS

From the Department of Human Anatomy, University of Oxford

THE accounts which have been published of the development of this region of the heart, the method in which the interventricular foramen is occluded to form the membranous part of the septum, or whether indeed this occurs at all, have been so vague or so contradictory that a re-examination of the question seemed worth while.

For this investigation I was able to study human embryos of 11·2, 11·4, 12·5, 14, 14·5, 15·5, 17, and 17·5 mm. in crown-rump length. Of these the 14 mm. embryo was the least valuable for my present purpose, as the heart is over-distended with blood in this specimen. I have therefore not included it among the detailed descriptions which follow. Reconstruction models were made of this region in the 11·2, 11·4, 14·5, 15·5, and 17 mm. specimens.

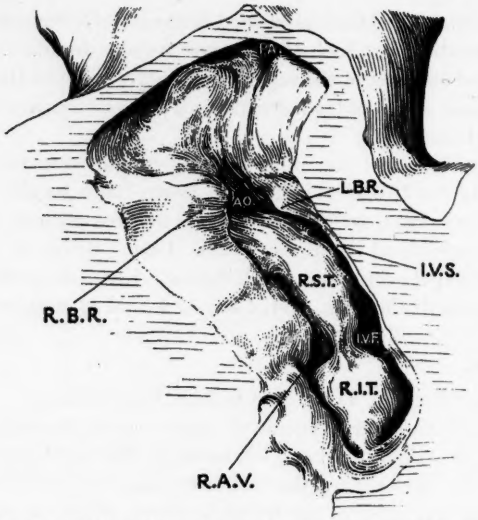
11·2 *mm. embryo.*

At this stage (Text-fig. 1) the two bulbar ridges (Frazer) or the proximal bulbar swellings (Tandler) are seen united above to form the bulbar septum, but soon separating below into right and left moieties. The right one (*R.B.R.*) can be traced down on the right ventricular wall to become continuous with the anlage of the right or inferior cusp of the tricuspid valve, while the left one (*L.B.R.*) joins the right side of the interventricular septum and on that soon disappears. Where they are fused and for a few sections after their separation the two ridges are seen to be of equal size, but at a lower level the right one soon appears to be not only the more extensive but the more substantial of the two.

The interventricular foramen (*I.V.F.*) is seen in thirty sections, i.e. its cranio-caudal diameter is 300 μ. It is bordered ventrally by the interventricular septum (*I.V.S.*) and dorsally by the fused auriculoventricular cushions (hereafter abbreviated into the A.-V. cushions). Above, the dorsal (cranial) border of the interventricular septum joins the fused A.-V. cushions about their centre, while below it joins their right margin.

Below the caudal border of the foramen the septum is fused to the inferior A.-V. cushion in fourteen sections (i.e. for 140 μ). This must presumably represent the amount of closure of the interventricular foramen which has already occurred since about the 7 mm. stage. It may be noted here, however, that in our 7·1 mm. embryo, where the interventricular septum is represented by a low ridge which does not yet make contact with the A.-V. cushions, the cranio-

caudal measurement of the interventricular foramen is only 220μ in contrast to 300μ in the specimen under consideration. This illustrates how difficult it is to compare accurately the condition of any single region in different embryos. There cannot be in the nature of things any constant initial size of foramen to be obliterated. Secondly, the foramen may not be diminishing steadily with the age of the embryo. Rather is it reasonable to suppose that it shares in the general growth and that its gradual closure is a compromise between two competitive processes.



Text-fig. 1. Drawing of a model of this region of the heart in an 11·2 mm. embryo (× 100) after removal of the ventral wall of the bulbus and right ventricle and of almost the whole of the interventricular septum, viewed from the right side. *R.B.R.* right bulbar ridge; *L.B.R.* left bulbar ridge; *R.S.T.* right superior tubercle of the A.-V. cushions; *R.I.T.* right inferior tubercle of the A.-V. cushions; *I.V.S.* interventricular septum; *I.V.F.* interventricular foramen; *R.A.V.* right auriculoventricular orifice; *A.O.* aortic channel; *P.A.* pulmonary artery.

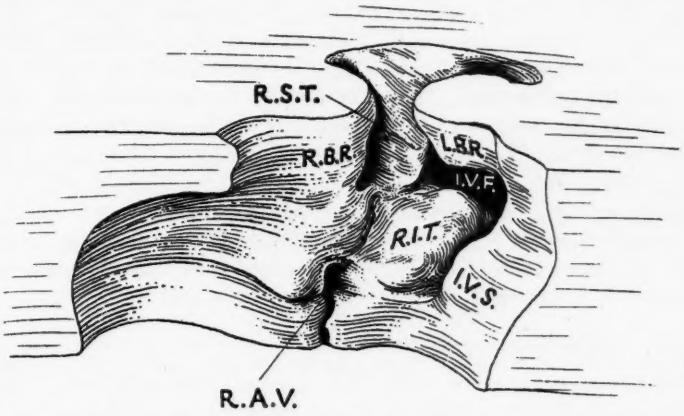
It is interesting to observe the method of closure here. In the three sections immediately below the foramen this is seen to be taking place by a narrow tongue-shaped proliferation of the right border of the A.-V. cushion making contact with and extending ventralwards on the right side of the septum (Pl. I, fig. 1). From the left margin of this proliferation contact with the septum gradually broadens, but there are ten sections below the foramen before the dorsal border of the interventricular septum and the A.-V. cushion are joined.

The right and left edges of the fused A.-V. cushions are seen to project as prominent swellings to form the medial boundaries of the right and left auriculoventricular orifices respectively. Further, in both these swellings superior and inferior tubercles can be distinguished corresponding presumably

to the original dorsal and ventral components of the fused cushions. Over the upper border of the right superior tubercle (*R.S.T.*), which is well cranial to the upper limit of the right auricular opening, the aortic channel passes to gain the left ventricle through the upper and dorsal portion of the interventricular foramen above the level of the lowest attachment of the left bulbar ridge to the interventricular septum.

11·4 *mm. embryo.*

I suspect that this embryo is older than its crown-rump length suggests. There is no record of its length before fixation, and in estimating this from an enumeration of the sections I expect an insufficient allowance was made for



Text-fig. 2. Drawing of a model of this region of the heart in an 11·4 mm. embryo (× 100) a ter removal of the ventral wall of the right ventricle and the greater part of the interventricular septum, viewed from the right side. *R.B.R.* right bulbar ridge; *L.B.R.* left bulbar ridge; *R.S.T.* right superior tubercle of the A.-V. cushions; *R.I.T.* right inferior tubercle of the A.-V. cushions; *I.V.S.* interventricular septum; *I.V.F.* interventricular foramen; *R.A.V.* right auriculoventricular orifice.

shrinkage. The model (Text-fig. 2) begins below the level of the bulbar septum and the two bulbar ridges appear quite separated. The left one (*L.B.R.*) is seen, as before, to join the interventricular septum well to the right side and ventral to its dorsal border. On this it soon disappears. The right ridge (*R.B.R.*) is the more prominent of the two and, as it is traced caudalwards, is found to become continuous with the projection of the commencing anterior cusp of the tricuspid valve. Between the two bulbar ridges are seen the marked elevations of the right tubercles of the fused A.-V. cushions, and now the separation between the superior (*R.S.T.*) and inferior (*R.I.T.*) is much more pronounced than in the earlier embryo.

The relation of the right bulbar ridge to these tubercles must be described

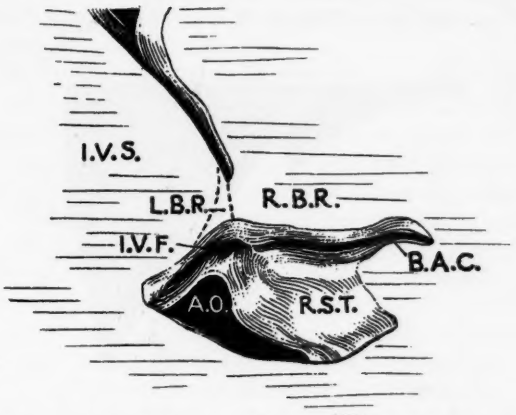
in some detail. While superiorly the two structures are quite separate, farther down the left margin of the right bulbar ridge gradually joins the right side of the lower portion of the superior and the upper portion of the inferior tubercle. In this way a part of the bulbar cavity is cut off and is found to be continuous below with that of the right auricle. This must mean that the right bulbar ridge has grown across the upper end of the right auriculoventricular orifice (*R.A.V.*) to meet the right A.-V. tubercles. In conformity with this, while the latter orifice measures 190 μ in its vertical extent in the 11·2 mm. embryo, it is now only patent for 40 μ. The interventricular foramen (*I.V.F.*) is very much smaller than in the earlier specimen. It is now only seen in eight sections, i.e. its craniocaudal diameter is only 80 μ. Caudal to its lower margin the ventricular septum and the inferior A.-V. cushion are fused for 320 μ in contrast to 140 μ in the first embryo. Further, a comparison with the 11·2 mm. specimen shows that the foramen is not only less extensive but is more obviously divided now into two parts. That portion of it cranial to and dorsal to the lower end of the left bulbar ridge is certainly forming the aortic channel, while the caudal and ventral part of it is being gradually obliterated. The closure of the foramen can be observed to be taking place here by the proliferation of the inferior tubercle in a cranial direction and it is this proliferation which makes the sulcus, separating the two tubercles, so much more pronounced (Pl. I, fig. 2). The microphotograph is that of the fourth section cranial to the foraminal closure and shows the rapidly extending cushion tissue bare of endocardium in contrast to the interventricular septum, which is still covered by it.

14·5 *mm. embryo* (Text-fig. 3).

The bulbar septum is now very much more extensive and forms the cranial border of the still patent interventricular foramen. The junction between its two component ridges can be distinguished as a more deeply staining seam, and by tracing this down it can be seen that it is the left bulbar ridge (*L.B.R.*) which joins the interventricular septum, as it does in the earlier embryos, but now it does so practically at its dorsal border and nothing like so much to its right side compared with the 11·4 mm. specimen. It soon fades out on the septum and does not appear to contribute any cushion tissue to it or to take any part in closing the foramen. The right bulbar ridge (*R.B.R.*), as it is traced caudalwards, is much the more extensive of the two. Just above the level of the interventricular foramen its left border is seen to be forming the greater part of the right wall of the aortic channel, while its right border bulges out prominently to form the anterior cusp of the tricuspid valve. Projecting into the aortic channel (*A.O.*) from its dorsal wall is the superior portion of the right tubercle of the A.-V. cushions (*R.S.T.*); it is impossible now to define accurately the division between superior and inferior tubercles.

The interventricular foramen (*I.V.F.*) is patent in four sections (20 μ in thickness). It is bounded now cranially, as has been said, by the bulbar septum, caudally by A.-V. cushion tissue, on its dorsal border by the right tubercle of the

A.-V. cushions (*R.S.T.*) and on its ventral border by the interventricular septum (*I.V.S.*). The microphotographs show how the foramen is being closed. In Pl. I, fig. 6, cushion tissue is seen proliferating from the A.-V. cushion, while a small disk-shaped mass of similar tissue caps the interventricular septum to the right of its dorsal border. In the next sections below this one this proliferation becomes more extensive until it reaches and fuses with the tissue capping the septum (Pl. I, fig. 7), so that the succeeding sections show a single fused mass joining the A.-V. cushion to the septum.



Text-fig. 3. Drawing of a model of this region of the heart in a 14·5 mm. embryo (× 100) viewed obliquely from above and from the left side. *R.B.R.* right bulbar ridge; *L.B.R.* left bulbar ridge; *R.S.T.* right superior tubercle of A.-V. cushions; *I.V.S.* interventricular septum; *I.V.F* interventricular foramen; *A.O.* aortic vestibule; *B.A.C.* bulbo-auricular channel.

The only interpretation which can be put on these appearances is that proliferating A.-V. cushion tissue is growing up from below on the ventral (septal) boundary of the interventricular foramen. These facts are shown in Text-fig. 4, which is a diagrammatic enlargement in silhouette of this region.

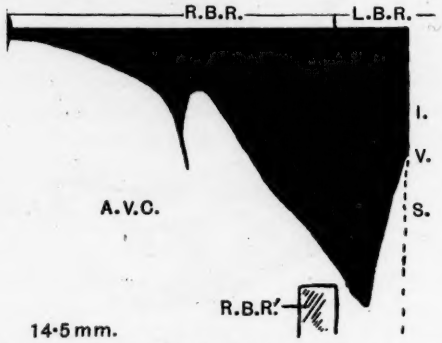
Further, Pl. I, fig. 7, shows that just at the caudal boundary of the foramen the left border of the right bulbar ridge is joining the tissue proliferating from the A.-V. cushion rather than the cushion itself as it was seen to do in the 11·4 mm. embryo. In either case, however, this junction is cutting off a bulbo-auricular channel which is far larger in the present specimen than in any of my others and which still communicates inferiorly with the right auricle.

12·5 *mm. embryo.*

In this specimen, while the interventricular foramen is more extensive, 180 μ in its vertical diameter, in some particulars the development of this region is more advanced. The right bulbar ridge and the superior tubercle of the A.-V. cushions, which seems to be poorly developed here, have blended

above. It is not until the level of the first section which shows the closure of the interventricular foramen that a narrow cleft is seen to be separating the two (Pl. I, fig. 3). When this narrow cleft is traced caudalwards through the succeeding four sections (40 μ) it is found to communicate with the right auricle and this is all that is seen of the much larger bulbo-auricular channel of the 14·5 mm. embryo.

A microphotograph of the next section below the first one (Pl. I, fig. 4) shows that the right bulbar ridge is in close coaptation at once with the right tubercle of the A.-V. cushions, with cushion tissue capping the interventricular septum, and with cushion tissue connecting them.



Text-fig. 4. *A.V.C.* A.-V. cushions from which tissue is proliferating to fill up from below the interventricular foramen, which is black, and to climb up on the dorsal border of the interventricular septum, *I.V.S.* *R.B.R.* right bulbar ridge joined with the left bulbar ridge, *L.B.R.*, to form the bulbar septum and the cranial boundary of the foramen. *R.B.R.'* is the lower margin of the right bulbar ridge at its junction with the proliferating A.-V. cushion tissue.

In the next three sections the connexion between the right tubercle and the cushion tissue capping the septum gradually seems to be more and more pronounced. They are still demarcated, however, from each other by a distinct notch on either side (Pl. I, fig. 5). This notch becomes indistinct in the course of the next few sections and then the A.-V. cushions appear as a uniform mass in contact with the interventricular septum. The origin of the horn-shaped cap of cushion tissue on the interventricular septum (Pl. I, fig. 3), which can be recognised in the three sections cranial to this level, might be thought to be doubtful from the appearances presented by the microphotographs (Pl. I, figs. 3–5). In this series it is seen to be in contact with the right bulbar ridge 10 μ rostral to the level at which it is connected with the right A.-V. cushion, but two facts make its origin from the latter certain. As it is traced caudalwards it obviously increases in size, which would surely be impossible if it were a derivative of the bulbar ridge. Further it is found to actually fuse with the right A.-V. cushion, while it is simply in coaptation with the right bulbar ridge, which does not appear to contribute anything to its formation.

I think therefore it can be truly regarded as having proliferated from the A.-V. cushions and the appearances presented here thus fall into line with those exhibited in the previous, 14·5 mm., specimen.

15·5 *mm. embryo.*

As in the 14·5 mm. embryo, the bulbar septum is seen here to form the cranial boundary of the interventricular foramen. The seam between its two constituents is distinct here, and the left bulbar ridge fuses with the dorsal border of the interventricular septum (Pl. II, fig. 8) and is contiguous with the cushion tissue which caps it (Pl. II, fig. 9). The foramen is now 30 μ in its craniocaudal diameter.

Above the level of the interventricular foramen the right bulbar ridge and the right superior tubercle of the A.-V. cushions are completely fused, and it is only at the caudal border of the foramen that a cleft appears which separates them (Pl. II, fig. 10). While this cleft separates the right bulbar ridge from the A.-V. cushion, it still permits the former to meet the cushion tissue which has proliferated to fill the foramen. As it is traced caudalwards the cleft gradually becomes wider and ends in the posterior portion of the right auriculoventricular orifice. The condition found here is almost exactly similar to that seen in the 12·5 mm. specimen and represents the remains of the bulbo-auricular channel of the earlier embryos, but owing apparently to a gradual extension cranialwards of the tricuspid orifice, the cleft is more correctly described now as communicating with that orifice rather than with the auricle.

In the left wall of the cleft immediately posterior to the junction of the right bulbar ridge and the cushion tissue capping the interventricular septum, a notch may be seen. This notch can be seen in seven sections (70 μ) caudal to the interventricular foramen and is in continuity above with its lower margin. It represents without doubt, I consider, the partial separation of the A.-V. cushion from the cushion tissue proliferated from it and is in the same situation as the notches referred to above in my description of the 12·5 mm. specimen.

17 *mm. embryo.*

At this stage it is becoming less easy to distinguish the different masses of cushion tissue, the bulbar septum and the A.-V. cushions.

The interventricular foramen is just completely closed, although in one section this is only effected by two strands of single cells, while in the section next below this the foramen is closed on either side with its central part still unobliterated. In both these sections the closing foramen appears as a cleft between two masses of cushion tissue. Above this level, as in the 15·5 mm. embryo, the right bulbar ridge and the superior tubercle of the A.-V. cushions are completely fused. At the level of the closing foramen, while they are still connected, they can be distinguished from each other, but in the next section below this a deep cleft divides them, as the right bulbar ridge separates off to enter into the formation of the right cusp of the tricuspid valve. For six

sections (120 μ) caudal to the closing foramen and in continuity with it a notch is seen on the left wall of the right ventricle, which partially separates a wedge-shaped mass of cushion tissue capping the interventricular septum from that of the A.-V. cushion (Pl. II, fig. 11). This is the same notch as that seen in the preceding specimen and, like it, represents the unobliterated portion of the groove between the A.-V. cushion and the cushion tissue which, springing from it, has grown up round the dorsal border of the interventricular septum. But, while in the earlier embryo it appears as a notch in the left wall of a discrete cavity, i.e. the caudal portion of the bulbo-auricular channel, it is now seen in the left wall of the posterior part of the right ventricle just above the upper limit of the tricuspid orifice. I think this difference can be explained in the following way. Owing to the enlarging ventricular cavity the connection between the right bulbar ridge and the right margin of the proliferation from the A.-V. cushions has become, as noted above, so much less extensive inferiorly that immediately below the level of the final closure of the interventricular foramen the previously discrete channel is thrown into that of the right ventricle by the disappearance of its anterior wall.

17·5 *mm. embryo.*

In this embryo the development of this region of the heart is now complete. The closure of the interventricular foramen is consolidated, and all the evidence one can see now for the processes I have described is a small indentation on the left wall of the posterior part of the right ventricle (Pl. II, fig. 12) which can be traced through nine sections (90 μ) and which is undoubtedly the remains of the more distinct notch observed in the earlier specimens.

The mass of cushion tissue capping the interventricular septum appears to be more obviously wedge-shaped than it does in the preceding photograph (Pl. II, fig. 11). Its configuration is due, of course, to the fact, to which attention has already been drawn, that the contact which the proliferating cushion tissue makes with the septum is asymmetrical. It joins it first to the right of and slightly ventral to its dorsal border and spreads subsequently as far as, but no farther than the latter point.

DISCUSSION

Collating my own observations I would say at once that while the upper portion of the interventricular foramen as seen in my 11·2 mm. specimen remains as the aortic channel, its lower portion is closed in the following way. At first the caudal portion of the right extremity of the inferior A.-V. cushion and the interventricular septum are approximated and fuse with each other to the right of the dorsal border of the septum. In both my 11·2 and my 11·4 mm. embryos this fusion appears to be effected by active proliferation of the cushion tissue (Pl. I, figs. 1, 2). In the later stages, as shown in my 12·5, 14·5 and 15·5 mm. specimens, there is, as I interpret it, some actual upgrowth of cushion tissue over the dorsal border of the interventricular septum, so that the final

closure is effected not, as in the earlier period, by apposition of the interventricular septum and the A.-V. cushion, but by the coalescence of the latter with proliferating cushion tissue derived from itself. Such a process as this is suggested by the microphotographs of the closing foramen (Pl. I, figs. 3–7) and also by the situation of the notch seen after the closure is complete in the 15·5, 17 and 17·5 mm. embryos (Pl. II, figs. 10–12).

I cannot say whether both the right A.-V. tubercles are involved in this obliteration of the foramen, because after the 11·4 mm. stage it is difficult to differentiate one from the other. The whole of the inferior tubercle certainly takes part in it and the upper portion of the superior one certainly does not, but one cannot be more precise than this.

With regard to the bulbar ridges, the left one is seen in the 11·4 mm. embryo to be attached at its lower end to the right side of the interventricular septum and thus to be demarcating the upper (dorsal) portion of the original interventricular foramen as the aortic channel from the lower (ventral) portion which is closed in the way I have just described. The level of the attachment of the left bulbar ridge to the septum seems to alter little. It is obviously difficult to obtain a fixed point in a rapidly developing organ like this, but, if the distance from the upper border of the right superior tubercle of the A.-V. cushions to the lower limit of the attachment of the left bulbar ridge to the septum is measured, this is found to be 50 μ in the 11·2 mm., 70 μ in the 11·4 mm. and 60 μ in the 14·5 mm. embryo. In the later 14·5 and 15·5 mm. specimens the two ridges have joined to form the bulbar septum, but the smaller left ridge can be still distinguished in the fused septum, and its inferior edge, as before, joins the interventricular septum. Here it forms the cranial boundary of the still patent foramen.

The right bulbar ridge has been shown in the 11·4 mm. embryo to grow across the upper portion of the right auriculoventricular orifice to reach the right border of the lower end of the right superior tubercle and the upper portion of the right inferior tubercle of the A.-V. cushions. In this way a portion of the original bulbar cavity is cut off, which communicates with the right auricle.

This bulbo-auricular channel is still patent in my 14·5 mm. embryo, but in my 12·5 and 15·5 mm. stages the upper portion of it has been closed by the approximation and fusion of the right bulbar ridge with the right superior tubercle of the A.-V. cushions and has been thus incorporated in the wall of the aortic vestibule. The lower end of the channel persists for a longer time. While this maintains its connexion with the right auricle up to the 14·5 mm. stage, thereafter, owing to the increasing extension cranialwards of the tricuspid orifice and the consequent decrease in the extent of the attachment inferiorly of the right bulbar ridge to the proliferation from the A.-V. cushions, it appears to be thrown into the posterior portion of the right ventricle in my 17 mm. embryo.

As far as I can ascertain, the above facts were first clearly observed by Frazer

(1916–17). He found that the track was closed at its aortic end in his 16 mm. specimen, while in his 18 mm. embryo no indication of its former position could be recognized with any certainty.

Do the bulbar ridges contribute anything to the closure of the interventricular foramen? If the right one is considered first, the lower portion of its left border was seen in the earlier stages (11·4 mm.) to join the ventral right margin of the tubercles of the A.-V. cushions. In the 12·5, 14·5, and 15·5 mm. embryos this border is connected not with the tubercles themselves, but rather with the cushion tissue proliferating from them to fill up the interventricular foramen, so that the notch, which I have already described as separating the A.-V. cushion from cushion tissue proliferating from it, is seen in the left wall of the bulbo-auricular channel (Pl. I, fig. 5; Pl. II, fig. 10). While the right bulbar ridge joins this proliferating cushion tissue, in no case does it appear to contribute anything to it, and it therefore cannot be said to take any part in closing the foramen. The result of this connexion, however, is that the anterior cusp of the tricuspid valve, which I believe is entirely formed from the free border of the right bulbar ridge, is continuous with the ventral end of the septal cusp when the development of the heart is completed.

The left bulbar ridge, where it joins the interventricular septum, is forming the cranial boundary of the interventricular foramen in my 14·5 and 15·5 mm. embryos, and must therefore fuse, as closure is completed, with A.-V. cushion tissue which has grown up to it along the dorsal border of the septum. Beyond this I cannot see that it helps in closing the foramen.

If it be objected that in the later stages, such as are shown in my 15·5 mm. embryo (Pl. II, figs. 8–10), the cushion tissue capping the interventricular septum might as well be interpreted as being derived from the left bulbar ridge growing downwards as from the A.-V. cushion growing upwards, I would maintain that the clue to its origin must be sought in the earlier specimens, where it has been shown clearly to be proliferating from the A.-V. cushions.

I have described the fate of the two bulbar ridges separately. Of course, when the 14·5 mm. stage is reached, their adjacent margins have completely joined with each other to form a perfect proximal bulbar septum, although the line of their coaptation can be distinguished as a seam even in the 17·5 mm. embryo.

The lower end of this septum is thus fastened eventually anteriorly to the interventricular septum and posteriorly to the right side of the A.-V. cushions and between these two points to the cushion tissue derived from their proliferation, which is filling up the foramen and which may thus be said to complete the lower margin of the bulbar septum.

In the descriptions of the closure of the interventricular foramen and the formation of the pars membranacea which have been published, different views have been expressed as to the relative importance of the part played therein by the interventricular septum, the proximal bulbar septum, and the A.-V. cushions. Born (1889), in his classical account of the development of the

rabbit's heart, wrote that the ventricular septum spreads upwards and unites gradually with the whole right margin of the fused A.-V. cushions and lastly with the right tubercle of the upper cushion. The lower margin of the bulbar septum unites with the free edge of the interventricular septum so that the remainder of the interventricular foramen is drawn into the aorta.

Mall (1912) described the interventricular foramen in his 11 mm. embryo as being bounded "above by the union of the septum aortopulmonale and the anterior (A.-V.) cushion, in front by the septum aortopulmonale, behind by the extended portion of the posterior cushion and below by the muscle of the ventricular septum". By the union of these different masses the foramen is closed and the membranous septum is formed.

Tandler (1912) gave no more detailed account than that of Mall. "The interventricular foramen becomes closed in the following manner. The proximal septum bulbi grows downward and reaches the septum interventriculare. The right end of the endocardial cushions, which have fused in the meantime, undoubtedly participate in the fusion of the two septa, but the extent of their participation cannot be exactly determined, since the entire circumference of the foramen interventriculare is surrounded by endocardial growths which pass into one another."

Frazer (1916–17, 1931) gave a much more precise, but very different, description of these points. According to him, the pars membranacea marks the position of the fused A.-V. cushions, but the interventricular foramen remains as the opening into the aortic vestibule from the general cavity of the left ventricle. By the growth of the two bulbar ridges, particularly of the left one, which spreads along the edge of the interventricular foramen, and by their subsequent fusion the aortic channel is roofed in and separated from the right cavity continuous with the pulmonary orifice. To quote from his original paper (1916): "There is no closure of the true interventricular foramen, for the structures concerned in the separation between the two channels reach the margin of the foramen and leave its lumen patent, affording passage to the blood from the left ventricle to the aortic channel."

I find it difficult to co-ordinate my description of the development of this region with Frazer's account of it. If Frazer's "true" interventricular foramen is the upper portion of the foramen above the level of the attachment of the left bulbar ridge to the interventricular septum, then all other accounts would agree that this does not close, but that it persists as the aortic vestibule. His figures, however, suggest that more than this of the original foramen remains patent, and not only does he not describe any filling up of the lower portion of it by proliferating A.-V. cushion tissue, but insists (1931) in his description of his 10 mm. embryo that the interventricular septum "has not grown upwards is evident from the fact that it retains its old level of attachment to the lower A.-V. cushion: this level is original and persistent throughout". Nor can I confirm his description of the left bulbar ridge extending along the free margin of the interventricular septum to reach its fellow. As I have mentioned already,

I found that the lower limit of its attachment to the septum remains fairly constant after the 11·4 mm. stage.

All other accounts of the development of this region regard the ventral portion of the pars membranacea as marking the site of the primitive interventricular foramen, and Frazer appears to be alone in denying this. While there is general agreement that the fused A.-V. cushions must form its posterior or upper portion, i.e. the part which separates the aortic vestibule from the right atrium (Hochstetter, 1898), Frazer is singular in making them responsible for the whole membranous part of the septum. My own observations would rather confirm, with the addition of some details, the classical description given by Born, who described the development of the rabbit's heart nearly fifty years ago.

CONCLUSIONS

The attachment of the left bulbar ridge to the interventricular septum may be said to divide the original interventricular foramen into two parts. That part of it, which is above and dorsal to this attachment, remains patent as the aortic channel; that part, which is below and ventral to it, is filled up by the active proliferation of the right tubercles of the fused auriculoventricular cushions to form the lower, ventral, half of the pars membranacea septi. The upper, dorsal, portion of the membranous septum is derived from the fused A.-V. cushions themselves.

The proximal bulbar septum is finally attached by its inferior border anteriorly to the interventricular septum, posteriorly to the right margin of the A.-V. cushions and between these two points to the cushion tissue which has proliferated from the latter to close the interventricular foramen, this proliferating tissue connecting the two bulbar ridges and so completing the bulbar septum inferiorly. While the bulbar septum is certainly responsible for the separation of the aortic from the pulmonary channel, it does not contribute anything to the occlusion of the interventricular foramen or to the formation of the pars membranacea septi.

I gratefully acknowledge my indebtedness to Prof. E. Fawcett and to Prof. D. M. Blair for their kindness in lending me embryos from their collections to amplify our own series here. I must also thank Mr W. Chesterman of this department for his assistance in the making of my models and for the preparation of the microphotographs.

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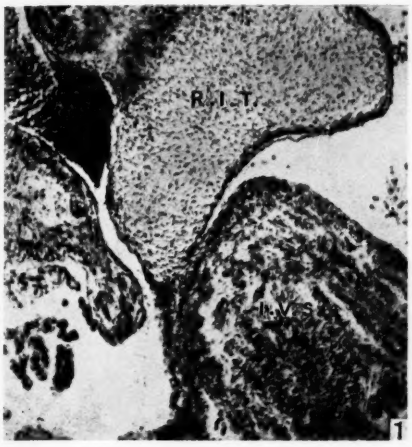
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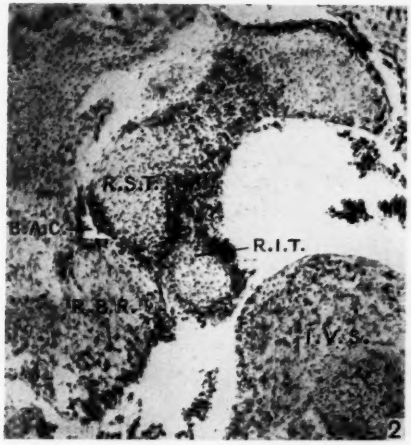
1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B.A.C.
R.I.T.
R.B.R.
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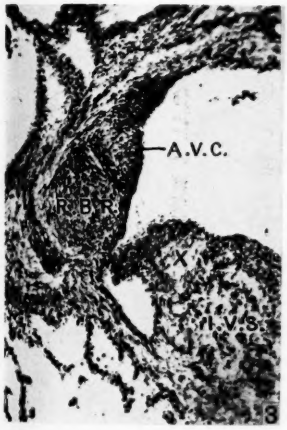
2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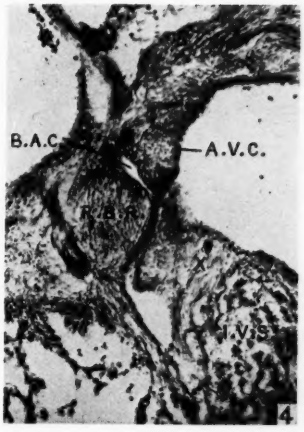
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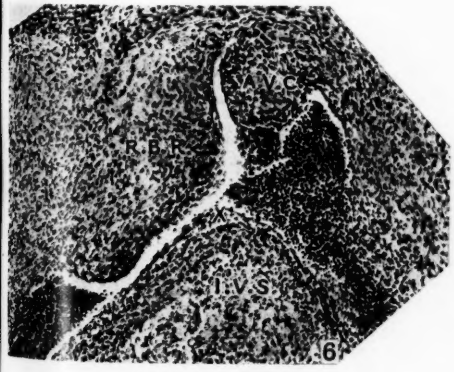
4

A.V.C.
I.V.S.
5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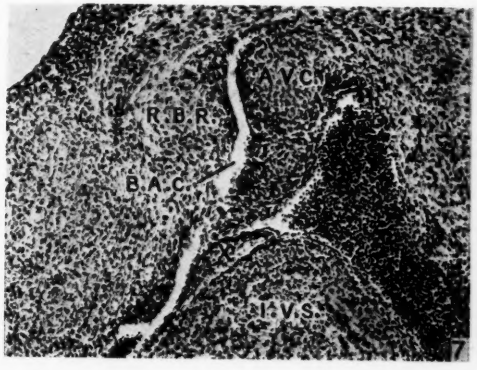
A.V.C.
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B.A.C.
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7

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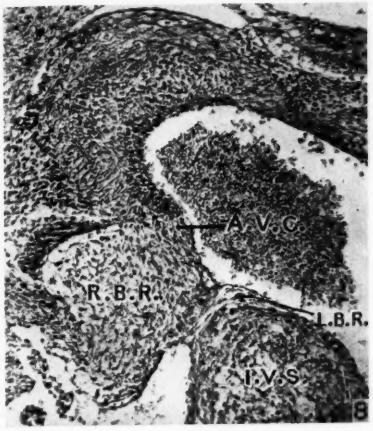
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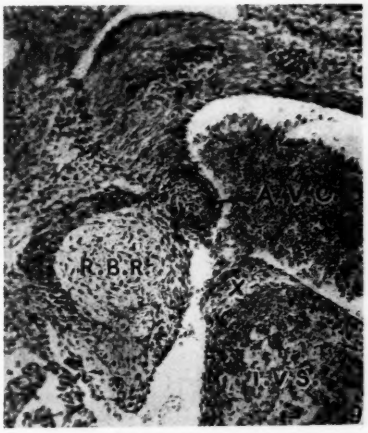
A.V.C.
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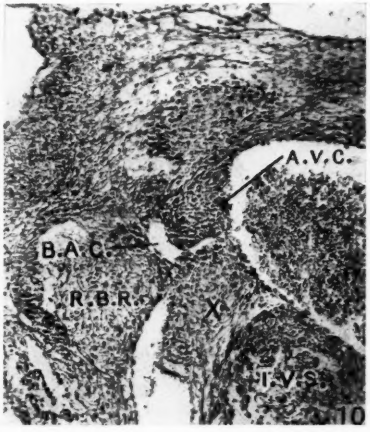
X
I.V.S.
9


A.V.C.
B.A.C.
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A.V.C.
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A.V.C.
X
I.V.S.
12

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EXPLANATION OF PLATES I AND II

Abbreviations

R.B.R. right bulbar ridge.
L.B.R. left bulbar ridge.
R.S.T. right superior tubercle of the A.-V. cushions.
R.I.T. right inferior tubercle of the A.-V. cushions.
I.V.S. interventricular septum.
A.V.C. auriculoventricular cushions.
B.A.C. bulbo-auricular channel.

PLATE I

Fig. 1. A section immediately caudal to the interventricular foramen in an 11·2 mm. embryo (× 80) to show that a tongue-shaped proliferation from the A.-V. cushion, *R.I.T.*, is making contact with the right side of the interventricular septum, *I.V.S.*, ventral to its dorsal border.

Fig. 2. A section through the interventricular foramen in an 11·4 mm. embryo (× 80). It shows the right bulbar ridge, *R.B.R.*, in contact with the right superior tubercle of the A.-V. cushions, *R.S.T.*, to form the bulbo-auricular channel, *B.A.C.* *R.I.T.* marks the inferior A.-V. tubercle proliferating cranialwards.

Figs. 3–5. Sections through this region of the heart in a 12·5 mm. embryo (× 80). Fig. 3 shows the interventricular septum, *I.V.S.*, capped by cushion tissue (*X*), which is in contact with the right bulbar ridge, *R.B.R.* In Fig. 4, which is the next section below Fig. 3, this tissue *X* is connected with both the right bulbar ridge, *R.B.R.*, and with the A.-V. cushions, *A.V.C.* Fig. 5 is a section three below the last and shows the proliferating tissue joining the A.-V. cushions, *A.V.C.* The junction between the two is marked on either side by a notch. The bulbo-auricular channel, *B.A.C.*, appears in Fig. 3 as a narrow cleft between the right bulbar ridge, *R.B.R.*, and the A.-V. cushions, *A.V.C.*: in Fig. 4 this channel is better marked, while in Fig. 5 it is seen to be continuous with the right auricle.

Figs. 6–7. These are sections through the heart of a 14·5 mm. embryo (× 80). In Fig. 6, which is through the centre of the interventricular foramen, the A.-V. cushion, *A.V.C.*, is seen to be proliferating, while similar cushion tissue, *X*, caps the interventricular septum, *I.V.S.* In Fig. 7 the proliferating cushion tissue is making contact with that on the septum, *X*, while its right border is joined by the right bulbar ridge, *R.B.R.*, as it encloses the bulbo-auricular channel, *B.A.C.*

PLATE II

Figs. 8–10. These are microphotographs of sections from a 15·5 mm. embryo (× 80). Fig. 8 is immediately cranial to the interventricular foramen and shows that in the proximal bulbar septum the right, *R.B.R.*, and left, *L.B.R.*, bulbar ridges can still be distinguished. The right one is fused with the right superior tubercle of the A.-V. cushions, *A.V.C.*, while the left one joins the interventricular septum, *I.V.S.* Fig. 9 is a section through the centre of the foramen and shows the septum, *I.V.S.*, capped with cushion tissue, *X*. The latter, as is seen in Fig. 10, which is the fourth section caudal to the level of the foraminal closure, joins the A.-V. cushion, *A.V.C.*, on its left side but is still separated from it on the right by a notch, which appears as a diverticulum from the anterior end of the inferior portion of the bulbo-auricular channel, *B.A.C.*

Fig. 11. This is a section through the heart of a 17 mm. embryo (× 80) five sections below the closing foramen. It shows the A.-V. cushion, *A.V.C.*, separated, as in the last figure, by a notch on the right side from the cushion tissue, *X*, which has proliferated from it to join the interventricular septum, *I.V.S.*

Fig. 12. This is a section from a 17·5 mm. embryo (× 80) and shows a notch in the same situation as that seen above, but now it is much less well marked.

THE EARLY STAGES OF REPLACEMENT OF THE SEMILUNAR CARTILAGES OF THE KNEE JOINT IN RABBITS AFTER OPERATIVE EXCISION[1]

By ROBERT WALMSLEY and JOHN BRUCE

Departments of Anatomy and Clinical Surgery, the University of Edinburgh

The changes which occur in the knee-joints of adult dogs after operative excision of the lateral semilunar cartilage have been recorded by the authors (1937), and at the same time certain clinical observations were made.

In a series of six adult dogs the lateral semilunar cartilage was replaced after its excision by a meniscus of fibrous tissue which had the general form of the excised cartilage. Some 5 months after the removal of the cartilage the regenerating mass (Pl. I, fig. 1) is a definite structure easily seen with the naked eye, but is not so thick nor so wide as the excised cartilage. One year after removal of the cartilage, however, the new meniscus has a transverse measurement greater than the normal cartilage, although, even at this time, it is not as thick as the normal. The new mass thus relatively soon attains, or even exceeds the normal cartilage in its surface area, a fact which is related, we believe, to the function of the cartilages.

The peripheral border of the regenerated meniscus is continuous in its entire extent with the articular capsule, but as a satisfactory examination of the replacing mass could only be made after the separation of the femur and the tibia, the exact origin of the new mass was difficult to determine. We have now performed a further series of experiments on the knee-joints of young rabbits to discover the exact origin of the new meniscus and the reaction of the related articular surfaces. These parts, it will be understood, can be observed only by the use of microscopic sections through entire joints. Young rabbits have been found to serve this purpose very well, as in addition to providing joints that are easily sectioned their medial semilunar cartilages are mobile—much more so than in man—and can be excised with the minimum of operative trauma.

OPERATIVE PROCEDURE AND HISTOLOGICAL METHODS

In view of the important clinical considerations the operative technique was substantially the same as that employed in the removal of the semilunar cartilages in the human subject.

[1] The expenses of this work were defrayed by a grant from the Earl of Moray Endowment Fund, Edinburgh University.

Under open ether anaesthesia an incision was made along the medial side of the patella and patellar ligament. The joint capsule was thereafter incised in the line of the skin incision, the edges of the capsular wound being retracted with fine forceps and the meniscus identified. A scalpel was inserted between the anterior end of the cartilage and the upper surface of the tibia, and carried laterally to divide the anterior horn. The anterior end of the cartilage was then grasped in forceps, the peripheral attachment of the cartilage divided, and the whole mass thereafter displaced by traction into the interior of the joint. The only remaining attachment—the posterior horn—was then cut across with a tenotomy knife.

The edges of the capsular incision were approximated by a running catgut suture and the skin closed with a continuous linen stitch.

After the operation the animals were allowed complete freedom of movement, and each of them, on the evening of the operation, was freely using the limb.

The first rabbit was killed 2 days after the operation and the others at intervals of 2 days after that. The knee-joint was isolated by dividing the bones about 2 cm. from the joint and it was then fixed in 10% formalin. Thereafter the blocks were decalcified in 3% nitric acid, embedded in paraffin, and sections 10μ thick were cut in the coronal plane and stained with haematoxylin and eosin.

The sections which have been found of most value in interpreting the process of formation of the replacing cartilages have been those passing through the region of the intercondylar eminence and it is photographs of these sections that have been reproduced.

EARLY CHANGES AFTER EXCISION OF CARTILAGES

In the normal knee-joint of the rabbit (Pl. I, fig. 2) the semilunar cartilages have the form and position which are typical of most mammals, and as is usual, the lateral cartilage is thicker than the medial. The articular cartilage of the femur and tibia is thickest over the parts of the bones which are not directly related to the semilunar cartilages.

A change in the articular capsule is seen 4 days after the operation. It consists of a proliferation of the fibrous tissue beneath the surface layer of the synovial membrane at the level of the plane of the knee-joint (Pl. I, figs. 3 and 4); the new cells are typical young fibroblasts. There is, at this time, also an increase in the thickness of the articular cartilage of the tibia where it is normally covered by meniscus, but the proliferation of cartilage cells is not uniform and there is an irregularity of the surface (Pl. I, fig. 3). During the next few days the proliferation of fibroblasts extends over all the synovial membrane, including the part of the membrane that is related to the non-articular parts of the bones. At the plane of the joint the proliferation now forms extensions inwards between the bones; these extensions are the begin-

nings of the new meniscus, which in the early stages is formed in two parts, an upper part which is related to the femur and a lower part related to the tibia (Pl. II, figs. 5, 6). In the following days the ingrowths grow rapidly and this early extension of the new meniscus over the articular surfaces suggests that the menisci are primarily related to the distribution of the synovial fluid on the articular surfaces rather than that they are weight-transmitting structures. The condition is analogous to that which was observed in dogs where the new meniscus first attains a surface area equivalent to or exceeding the normal and only secondarily attains the normal thickness.

About 20 days after the excision, the two parts of the meniscus fuse with each other to form a single mass, but as the fusion is secondary to the increase in thickness of the peripheral part of the meniscus the time at which it takes place varies within fairly wide limits, as the rate of growth is by no means constant in different rabbits.

During the period of active growth the new meniscus is much more cellular than the normal semilunar cartilage (Pl. II, figs. 8 *a*, *b*) and throughout this period there are no cartilaginous cells in any part of it; it is formed only of actively proliferating fibrous tissue cells, and these have been derived from the synovial stratum of the articular capsule of which the menisci are morphological parts.

The attachments of the new meniscus differ from the typical lateral semilunar cartilage not only in that there is no bony attachment by anterior and posterior horns, but also in the wide and continuous attachment of its peripheral margin to the articular capsule and the non-articular parts of the femur and tibia immediately adjacent to the articular cartilage.

SUMMARY

1. Replacement of the semilunar cartilages invariably takes place after their complete removal.

2. The replacing mass is derived from the synovial layer of the articular capsule.

3. The replacing mass is composed, in the early stages at least, of fibrous tissue and fibrous tissue alone.

4. Replacement begins within a very few days and the replacing mass very soon has the form and surface area of the removed disk.

We should like to express our thanks to Prof. J. C. Brash for his help and guidance and to Prof. Sir D. P. D. Wilkie for facilities afforded by his Department.

REFERENCE

Bruce, J. & Walmsley, R. (1937). *Brit. J. Surg.* vol. xxv, p. 17.

Fig. 1.

Fig. 2.

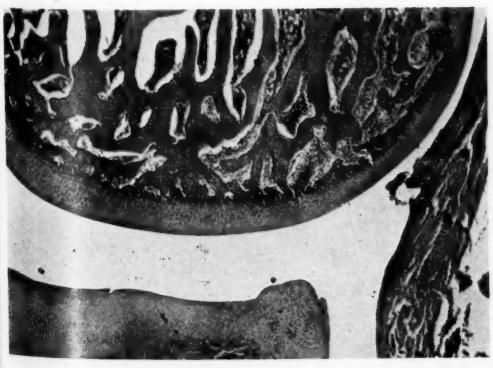

Fig. 3.

Fig. 4.

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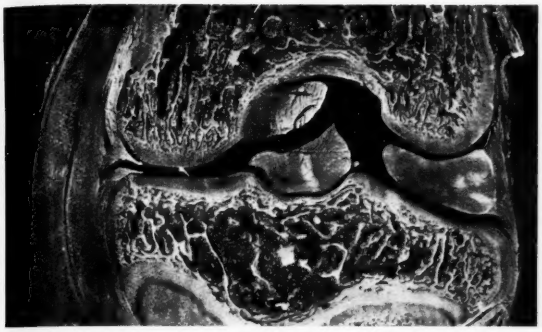

Fig. 5.

Fig. 8*a*.

Fig. 6.

Fig. 8*b*.

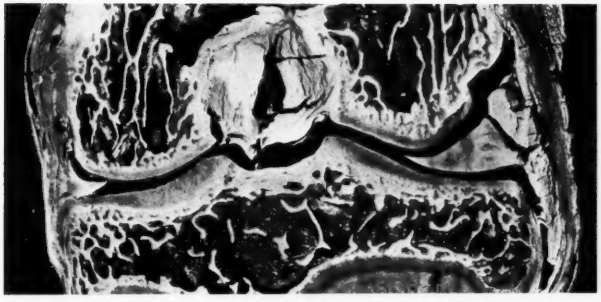

Fig. 7.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Upper surfaces of right and left tibiae of dog 5 months after the removal of the right lateral semilunar cartilage. The new meniscus is neither so thick nor so wide as the normal left lateral cartilage.

Fig. 2. Coronal section of normal knee-joint of young rabbit.

Fig. 3. Coronal section of knee-joint of young rabbit 4 days after the removal of the medial semilunar cartilage. The dark area in the articular capsule at the level of the plane of the joint is the region of the cellular proliferation in the synovial membrane.

Fig. 4. Synovial membrane of same specimen as fig. 3 to show the proliferation of fibroblasts at the level of the plane of the joint.

PLATE II

Fig. 5. Coronal section of knee-joint of rabbit 10 days after removal of the medial semilunar cartilage. The new meniscus has distinct upper and lower parts both of which are continuous with the articular capsule.

Fig. 6. Same specimen as shown in fig. 5 to show the continuity of the new meniscus and the articular capsule. In this figure the tibia is uppermost.

Fig. 7. Coronal section of knee-joint of rabbit 22 days after the removal of the medial semilunar cartilage. The new meniscus in this specimen is a single structure, the upper and lower parts having fused.

Fig. 8 *a*, *b*. (*a*) Illustrates the central part of a normal medial semilunar cartilage, and its relative non-cellularity is to be contrasted with (*b*) which shows the replacing mass 20 days after the excision of the medial cartilage.

THE EFFECTS OF PROLONGED OESTROGENIC STIMULATION ON THE PROSTATE OF THE RHESUS MONKEY

By S. ZUCKERMAN

From the Department of Human Anatomy, Oxford

Observations reported elsewhere (Parkes & Zuckerman, 1935; Courrier & Gros, 1935; van Wagenen, 1935) suggest that the prostatic glands of macaque monkeys do not react to oestrogenic stimulation like those of mice (Lacassagne, 1933; Burrows, 1935 *a*, *b*) and dogs (de Jongh & Kok, 1935; Zuckerman & Groome, 1937). In non-primate mammals the glandular epithelium undergoes both hyperplasia and metaplasia, and the glandular acini are transformed into cysts of various size filled with desquamated epithelium. On the other hand the glandular epithelium remains unchanged in rhesus monkeys which have been injected with oestrone for as many as 89 days (Zuckerman & Parkes, 1936). This difference was made part of the basis of an embryological interpretation of the changes induced by oestrogens in the male reproductive tract (Zuckerman, 1936*a*). The possibility that the experimental data on different mammals were not strictly comparable because male monkeys had not been treated with oestrin for sufficiently prolonged periods made it necessary to obtain further experimental data on the question.

EXPERIMENTAL

Two castrated immature rhesus monkeys (*Macaca mulatta*) were injected intramuscularly with an oil solution of oestrone for 368 (O.M. 115) and 365 (O.M. 120) days respectively. O.M. 115 received 100γ a day. O.M. 120 was given 10γ daily for the first 34 days, 5γ daily from day 35 to day 45, and 2·5γ daily from then onwards. The maximum and minimum weights of the two animals during the experimental period were:

O.M. 115	1720 g.	2940 g.
O.M. 120	2160 g.	3140 g.

Both animals showed the usual external responses to the injections, those of O.M. 115 being the more pronounced. At no time did either animal appear to be suffering from urinary retention. The reproductive organs were removed on the day following the last injection, and fixed in Bouin's fluid. The prostates were cut serially, the sections being stained with Mayer's haemalum and eosin.

O.M. 115. The changes in the reproductive tract of this animal are in general no different from those that occur after shorter periods of oestrogenic stimulation. The true prostatic glands are unchanged, and considerable growth has taken place in the vagina masculina, in the terminal parts of the ejaculatory ducts and in the fibro-muscular stroma within which they lie.

The whole prostate is much enlarged. Pl. I, figs. 1 and 2 represent transverse sections taken through the level of the middle of the vagina masculina of the prostate of O.M. 115, the experimental animal, and of O.M. 39, a control animal, of the same age, that had not been injected. Although the total transverse area of the prostate of O.M. 115 is almost three times as great as that of O.M. 39, the area within which the true prostatic glandular tissue lies is approximately the same in both (cf. Tables I and II). The great increase in the size of the prostate of O.M. 115 is due mainly to growth in the fibro-muscular stroma that intervenes between the urethra and the part of the organ in which lie the true prostatic glands. Through this stroma, to which the name "utricular bed" may be applied, pass the ejaculatory ducts and the vagina masculina.

Although the total glandular area does not appear to be smaller than in normal controls, the glands themselves are reduced in number and more dispersed than usual, the relative amount of fibro-muscular tissue within the glandular region being greater than in normal specimens of comparable age. The epithelium of the prostatic glands proper is unchanged (Pl. II, fig. 7). The glandular epithelium in the part of the organ referred to by van Wagenen (1936) as the coagulating gland is in general also unchanged (Pl. II, fig. 6) and is tall columnar and in a few places two- or three-layered. On the other hand the epithelium of the main collecting ducts of most of the prostatic glands has undergone metaplasia. Except in the uppermost lobules of the so-called "coagulating gland", the change in their character is confined to those parts of the ducts lying within the utricular bed. In general the change is more pronounced in the distal (urethral) parts of the ducts. The metaplastic process has followed the usual course, the normal one or two layers of cylindrical cells being transformed into a heavily stratified and desquamating epithelium. In places, the ducts, many of which open into the terminal parts of the ejaculatory ducts (Pl. II, fig. 5), appear to be transformed into solid rods of cells, and in more cranial levels of the prostate some are more conspicuous structures than the vagina masculina itself.

Similar metaplasia has occurred in the terminal parts (approximately the distal 2·2 mm.) of the ejaculatory ducts. The left duct, into which the vagina masculina opens, is more affected than the right and is considerably distended above the level where stratification ceases, its maximum diameters being 6 by 3 mm. It is not unlikely that the great distension of this duct resulted from the narrowing of the distal part of its lumen. The thickened walls of the two ejaculatory ducts are joined together just cranial to the level at which they open by a common mouth into the urethra (Pl. II, fig. 5).

The vagina masculina opens into the left ejaculatory duct immediately above the point where the latter enters the urethra. It is approximately 5·5 mm. long and extends to within 2 mm. of the uppermost level to which prostatic glands (coagulating glands) reach. The cranio-caudal diameter of the whole prostate is approximately 11 mm. The wall of the vagina masculina is very thick and is composed of a many-layered stratified epithelium from whose basal layers numerous tongue-like projections of cells project into the utricular bed. The superficial layers of the utricular cells were apparently being rapidly shed, a process which also occurs during less prolonged periods of oestrogenic stimulation.

From the region of the opening of the ejaculatory ducts to the external meatus the urethral epithelium is considerably thickened, the superficial layers of the stratified epithelium being "cornified". Numerous mitotic figures are to be seen in the chromophilic basal zone of the urethral epithelium. The greatest amount of growth had taken place in the epithelium of the dorsal part of the prostatic urethra, the epithelium forming the ventral wall in this region being practically unchanged. A small conical process projects cranially into the urethra from the thickened epithelium of the crista urethralis.

Many leucocytes are to be seen in the thickened urethral epithelium. Collections of lymphocytes are also very common. They mainly occur just deep to the urethral epithelium, the "nodes" increasing in frequency and size from the proximal part of the penile urethra to the opening of the urethra into the bladder. Thus lymphocytic "nodes" entirely surround the upper prostatic urethra (Pl. I, fig. 2). Similar collections of lymphocytes occur within the utricular bed (Pl. I, fig. 2) and in some of them areas of breakdown are to be seen. Many of the "nodes" are large enough to be obvious to the naked eye. Some appear to have begun by surrounding thickened collecting ducts. Except in a few of the most cranial lobules of the prostate ("coagulating gland") lymphocytic collections do not occur in that part of the organ within which the true prostatic glands are situated.

The walls of the lobules of the seminal vesicles are considerably thickened. The epithelium of the vesicles on the other hand is much simpler than usual and in general comprises a single unfolded layer of cuboidal cells. In no region of the seminal vesicles is there any evidence of epithelial hyperplasia or metaplasia.

O.M. 120. The amount of oestrone injected into this animal was insufficient to cause any obvious epithelial changes in the prostate. The prostatic glands and collecting ducts are normal, and no growth has taken place in the terminal parts of the ejaculatory ducts. The vagina masculina is abnormal in structure and comprises a system of somewhat diffusely scattered cords of cells which end cranially in a cyst. Numerous solid tongue-like projections from the wall of this cyst extend into the utricular bed. Similar projections pass from the solid cords which connect the cyst with the crista urethralis. The urethral epithelium is normal throughout its entire extent. The few suburethral col-

lections of lymphocytes that are present are situated in the upper part of the prostate.

The only clear evidence that the prostate of this animal has been influenced by the injections of oestrone is given by the fact that the gland is larger than normal and by the fact that the proportion of glandular tissue to fibro-muscular stroma is much less than in normal animals of comparable age (Tables I and II). The growth has mainly affected the utricular bed (see below).

The seminal vesicles of this animal exhibit changes similar to but slightly less extensive than those shown by O.M. 115.

FURTHER OBSERVATIONS AND DISCUSSION

The findings reported above confirm the view that the prostatic glands of rhesus monkeys are unresponsive to oestrogenic stimulation. The glands presumably either develop from epithelium of the urogenital sinus which is unresponsive to oestrogens, or they become unresponsive after they have budded from the sinus.

The enlargement of the prostate in non-primate mammals as the result of oestrogenic stimulation is in large part due to hyperplasia and metaplasia of the epithelium of the true prostatic glands. The view that benign enlargement of the prostate in man is also a result of oestrogenic stimulation, as formulated by those who have investigated non-primate mammals only (e.g. Burrows, 1935*b*), is based on the belief that the prostatic glands in man would react in the same way as do those of non-primate mammals. This belief is hardly tenable in view of the facts reported above, for it is unlikely that the human prostate will react more like that of a mouse than like that of a monkey. The fact remains, however, that the primate prostate does increase in size as the result of oestrogenic stimulation, and it is necessary to analyse the nature of this growth.

Tables I and II give the total area (*a*) and the separate areas of (*b*) the glandular part, (*c*) the utricular bed (as defined above) and (*d*) the urethral part of transverse sections of the prostates of normal rhesus monkeys (Table I), and of rhesus monkeys that had been injected with oestrone (Table II). In each case the section measured was taken at a level through the middle of the vagina masculina. The sections were projected on to squared paper and the glandular area (see Pl. I, figs. 1 and 2), the utricular bed and urethra (Pl. I, fig. 2), drawn and measured. In only one case (O.M. 36) was it not possible to demarcate the urethral from the utricular area by the limits of its circular muscle. In eight of the twenty-four specimens listed in Tables I and II the glandular area forms a compact mass as in Pl. I, fig. 1. In the remaining sixteen it is broken up into separate lobules (Pl. I, fig. 2). All the specimens had been fixed and prepared in the same way. The animals have been aged according to the tables for body weight and dentition published by Schultz (1933, 1935).

The extent of the prostatic growth which occurs as a result of the injection of oestrone can be realized by comparing the total areas of the specimens listed in Table II with those of comparable age in Table I. The increase becomes apparent after as few as 14 days of injection (O.M. 36) and is considerable after a year's treatment (O.M. 115). Presumably an even greater increase would be indicated if it were possible to obtain a three-dimensional instead of a two-dimensional view of the growth-process.

Table I. *Dimensions of mid-utricular transverse sections of the prostates of normal rhesus monkeys. For explanation see text*

		(*a*)	Glandular area (*b*)		Utricular bed (*c*)		Urethra (*d*)		(*c* + *d*)	
No. O.M.	Age months	Whole organ actual sq. mm.	Actual sq. mm.	%	Actual sq. mm.	%	Actual sq. mm.	%	Actual sq. mm.	%
112	18	21·8	13·6	62·5	2·3	10·6	5·9	26·9	8·2	37·5
125	20	22·0	11·7	53·3	1·3	5·9	9·0	40·8	10·3	46·7
8	20	25·3	13·4	53·0	2·9	11·7	8·9	35·3	11·8	47·0
38	24	28·6	16·3	56·9	1·5	5·2	10·8	37·9	12·3	43·1
39	28	31·0	19·2	61·9	1·4	4·5	10·4	33·6	11·8	38·1
13	32	21·8	9·5	43·7	2·6	11·8	9·7	44·5	12·3	56·3
164	33	21·4	13·2	61·4	3·1	14·6	5·1	24·0	8·2	38·6
158	34	30·9	17·9	58·0	2·2	7·2	10·8	34·8	13·0	42·0
140	36	29·6	15·5	52·5	3·0	10·1	11·1	37·4	14·1	47·5
163	40	58·1	43·7	75·2	4·1	7·1	10·3	17·7	14·4	24·8
182	44	28·2	18·0	64·0	2·1	7·4	8·0	28·6	10·1	36·0
157	46	64·1	43·9	68·5	3·6	5·6	16·6	25·9	20·2	31·5
90	50	45·7	24·0	52·5	3·4	7·4	18·3	40·1	21·7	47·5
149	Mature	216·9	182·8	84·3	4·7	2·2	29·4	13·5	34·1	15·7

Table II. *Dimensions of mid-utricular transverse sections of the prostates of rhesus monkeys that had been injected with oestrone. For explanation see text*

				(*a*)	Glandular area (*b*)		Utricular bed (*c*)		Urethra (*d*)		(*c* + *d*)	
No. O.M.	Age months	Oestrone daily I.U.	Days	Whole organ actual sq. mm.	Actual sq. mm.	%	Actual sq. mm.	%	Actual sq. mm.	%	Actual sq. mm.	%
155	16	1000	37	42·4	17·5	41·1	18·0	42·4	7·0	16·5	24·9	58·9
153	19	1000	22	42·5	21·7	50·5	12·1	28·2	8·7	21·3	20·8	49·5
151	22	1000	37	37·6	19·1	50·7	12·4	32·9	6·1	16·4	18·5	49·3
36	28	500	14	41·2	22·6	55·0	—	—	—	—	18·5	45·0
115	28	1000	368	82·6	20·9	25·3	43·5	52·6	18·2	22·1	61·7	74·7
79	30	1000	15	50·3	20·9	41·6	14·6	29·0	14·8	29·4	29·4	58·4
101	30	1000	62	51·1	14·3	28·0	14·9	29·3	21·9	42·7	36·8	72·0
148	32	1000	15	68·6	34·9	50·9	12·0	17·5	21·7	31·6	33·7	49·1
120	33	25	365	45·4	15·2	33·5	14·7	32·4	15·5	34·1	30·2	66·5
24	36	500	14	82·5	55·2	67·3	6·3	7·6	21·0	25·1	27·3	32·7

Table I suggests that the absolute area of glandular tissue remains relatively constant until the fourth year of life, and that a considerable increase occurs by the time maturity is reached. Even in the earlier stages, however, the glandular region as a rule comprises more than 50 per cent of the total transverse area of the prostate. The proportion becomes greater as maturity is

reached. The absolute area of the utricular bed on the other hand increases very little, and decreases relatively as the prostate grows. In no instance does the utricular bed comprise more than 15 per cent of the transverse area of the prostate, and it is usually less than 10 per cent. The urethral area, on the other hand, appears to increase gradually, so that its relative area does not decrease until full maturity is reached.

Table II shows that the relative extent of the glandular area decreases considerably as a result of prolonged oestrogenic stimulation, although the actual glandular area increases somewhat. Since the glands themselves are more widely dispersed in the prostates of animals that have been injected with oestrone, it follows that growth in the glandular area is due mainly to an increase in its fibro-muscular elements. The urethral part also increases in actual area as a result of oestrogenic stimulation, but its relative area does not increase. The main growth in the prostate is plainly in the utricular bed, in which there is both a considerable absolute and a considerable relative increase in size. Thus the greatest actual area of a utricular bed in the control series is 4·7 sq. mm. (O.M. 149) and the greatest relative area 14·6 per cent (O.M. 164), whereas the greatest absolute area in the injected series is 43·5 sq. mm. (O.M. 115) and the greatest relative area 52·6 per cent (O.M. 115). The tables also give the dimensions of the utricular bed together with the urethral part of the prostate, and this measure also indicates clearly the nature of the changes which occur as a result of oestrogenic stimulation ($c + d$).

The various relations between the parts of the prostate in the normal and in the injected series of animals are shown diagrammatically in Text-figs. 1–4. Text-fig. 1*b* shows the mean actual proportions of the parts of the prostate in the ten experimental animals listed in Table II. Text-fig. 1*a* shows the mean actual proportions, drawn to the same scale, of the parts of the prostate in the nine normal animals of comparable age listed in Table I. Text-fig. 2 shows the mean relative proportions of the parts of the prostate in the same two groups of animals. Text-fig. 3*b* shows the actual proportions of the parts of the prostate of O.M. 115, the animal that had been injected within oestrone for a year. Text-fig. 3*a* shows the corresponding proportions, drawn to the same scale, in O.M. 39, a normal control of comparable age. Text-fig. 4 shows the relative proportions of the parts of the prostate in these two animals. These diagrams very clearly demonstrate the great increase that takes place in the size of the utricular bed under the influence of oestrone, and the relative decrease that occurs in the glandular area.

It is plain that as the prostate increases in size under the influence of oestrogenic stimulation, the true prostatic glandular tissue is pushed outwards by growth in the utricular bed so that it comes to form an investment to the organ (cf. Pl. I, figs. 1 and 2). One of the widely held clinical views concerning the aetiology of benign enlargement of the prostate in man is that adenomata develop in the so-called middle prostatic lobe and by their growth displace the ejaculatory ducts towards a "false capsule" of the organ that is

formed by the compressed true prostatic glands. In favour of this view is the well-known fact that when an enlarged prostate is carefully enucleated the ejaculatory ducts are usually left uninjured. The origin of the adenomata has given rise to several theories. Recently the view has been put forward in a series of papers (Zuckerman & Parkes, 1935, 1936; Zuckerman, 1936*a*, 1936*b*) that the *epithelial* changes in benign enlargement constitute hyperplasia in

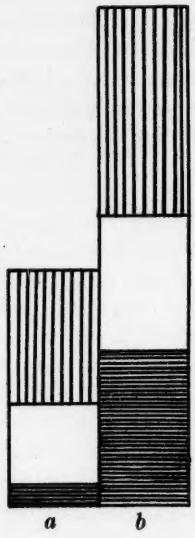

Fig. 1.

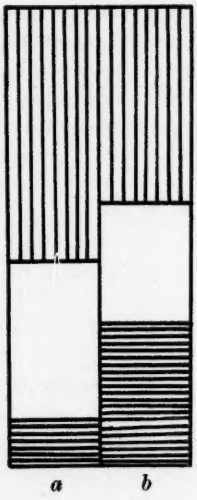

Fig. 2.

Text-fig. 1. *a*, the mean actual proportions of the parts of the prostate in the first nine normal control animals listed in Table I; *b*, the mean actual proportions of the parts of the prostate in the ten experimental animals listed in Table II, drawn to the same scale. Horizontally lined area = utricular bed. Clear area = urethral part. Vertically lined area = glandular part.

Text-fig. 2. *a*, the mean relative proportions of the parts of the prostate in nine normal control animals; *b*, the mean relative proportions of the parts of the prostate in the ten experimental animals. Different areas as in Text-fig. 1.

response to oestrogenic stimulation of a true uterine rudiment of the Müllerian ducts. The view that the uterus masculinus is primarily concerned in the pathological process has since been restated by Cunéo (1936). The hyperplasia of a glandular system lying within a stroma which reacts to oestrogenic stimulation as intensely as does the utricular bed of monkeys would undoubtedly produce a structural alteration in the prostate indistinguishable from that of benign enlargement.

It is of great interest, as the results of the experiment with O.M. 120 clearly show, that the fibro-muscular tissue of the utricular bed and of the urethral part of the prostate is much more sensitive to oestrogenic stimulation than any epithelial tissues within the prostate. It is also more sensitive than the stroma of the glandular region.

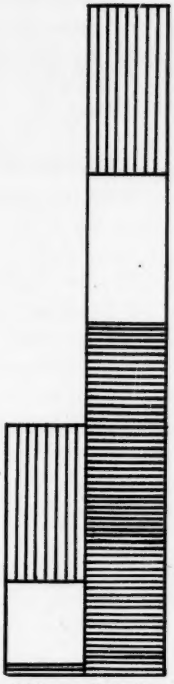

Fig. 3.

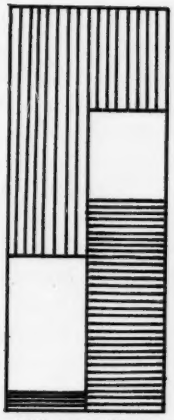

Fig. 4.

Text-fig. 3. The actual proportions of the parts of the prostate in O.M. 39, a normal control animal, and in O.M. 115 the experimental animal of the same age, drawn to the same scale.

Text-fig. 4. The relative proportions of the parts of the prostrate in O.M. 39 and O.M. 115. Different areas as in Text-fig. 1.

The metaplasia that occurs in the ejaculatory ducts raises a difficult problem. The change is restricted to the epithelium of the distal parts of the ducts. That of the seminal vesicles does not undergo growth in response to oestrogenic stimulation. The ejaculatory ducts, however, like the seminal vesicles, are derivatives of the Wolffian system, the epithelium of which would not *a priori* be expected to react to oestrogenic stimulation. The collecting ducts are derivatives of sinus epithelium, and, unlike the ducts themselves,

their distal extensions, which comprise the prostatic glands proper, are unresponsive to oestrogenic stimulation.

I have elsewhere (1936*a*) suggested that epithelial metaplasia and stratification in response to oestrogenic stimulation may in general be a primary response of tissue in whose development oestrogen-sensitive sinus epithelium has played a part. This hypothesis suggests that the original epithelium of the ejaculatory ducts is replaced by oestrogen-sensitive sinus epithelium. According to some authorities, e.g. Vilas (1932), a corresponding replacement of Müllerian by sinus epithelium occurs in the human vagina, and this view, as is pointed out elsewhere (Zuckerman, 1936*a*), is abundantly supported by endocrinological evidence. That there is a general tendency for the epithelium of the urogenital sinus of the monkey to extend in a cranial direction is indicated by the frequency of cranially directed diverticula from the prostatic urethra. Shallow pockets are by no means uncommon, and the following examples represent only the more obvious instances of such diverticula.

O.M. 115, Pl. II, fig. 1. The vagina masculina opens into the left ejaculatory duct. Vintemberger (1926) states that such an arrangement occurs in 8 per cent of cases in man. According to Bloomfield & Frazer (1927) the Wolffian and Müllerian ducts in man remain separate in their entire extent and open close together into the urogenital sinus. To my knowledge there is no evidence which points to an opposite conclusion in other Old World Primates, and consequently it follows that the distal part of at least the left ejaculatory duct of O.M. 115 represents an evagination or diverticulum of the urogenital sinus into which both the terminal part of the genital cord (vagina masculina) and the left Wolffian duct (vas deferens) open.

O.M. 101, Text-fig. 5. The two ejaculatory ducts (*E*) and vagina masculina (*V*) join together before they open by a common mouth into the prostatic urethra (*U*). For the same reasons given above, it would seem that the common opening represents an upward extension of the urogenital sinus.

M.M. 20, Text-fig. 6. On both sides a urethral diverticulum (*UD*) passes dorsally and cranially into the prostate. That on the left side is shown as a solid block of cells. That on the right has a patent lumen and is shown connected to the urethra by a strand of cells. The ejaculatory ducts (*E*) and vagina masculina (*V*) open into the urethra in the normal manner.

M.M. 24, Text-fig. 7. Two urethral diverticula (*UD*) are shown lying between the urethra (*U*) on the one hand and the vagina masculina (*V*) and ejaculatory ducts (*E*) on the other.

O.M. 21, Text-fig. 8. A right accessory ureter (*AU*) opens into the cranial part of a blind urethral diverticulum (*UD*) which passes upwards in the crista urethralis. The diverticulum is about 1·25 mm. long, and just before it opens into the urethra it is joined by a very narrow tubular diverticulum which opens further caudally into the prostatic urethra at about the same level as the ejaculatory ducts (*E*). The vagina masculina (*V*), which is about 4·6 mm. long, opens into the distal part of the prostatic urethra about 0·7 mm. more caudally.

Fig. 5.

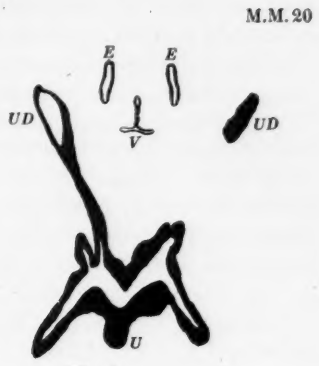

Fig. 6.

Fig. 7.

Fig. 8.

Text-figs. 5–8. Tracings of projections of the prostates of four monkeys, demonstrating urethral diverticula. The varying thickness of the outlines indicates varying responses to oestrogenic stimulation. The urethra of O.M. 21, Text-fig. 8, is incomplete in the section and has been indicated by interrupted lines. *E*, ejaculatory duct; *V*, vagina masculina; *U*, urethra; *UD*, urethral diverticulum; *AU*, accessory ureter.

I have observed similar urethral diverticula in marmosets (*Hapale jacchus*). Thus large dorsally and cranially directed diverticula occur in two of three specimens, M.M. 69 and 71 (Pl. I, figs. 3 and 4). In one specimen the uterus masculinus opens into the diverticulum.

The relative frequency of urethral diverticula of the kind described above suggests that there is a tendency for sinus epithelium to track cranio-dorsally in the region of the opening of the Wolffian and Müllerian ducts. Such a view is consonant with Vilas' (1932) and Koff's (1933) findings regarding the development of the vagina. It is not being unduly speculative, therefore, to suggest that the epithelium of the distal parts of the ejaculatory ducts which undergoes metaplasia in response to oestrogenic stimulation represents oestrogen sensitive sinus epithelium that has replaced the original Wolffian epithelium.

The different reactions to oestrone of the prostatic glands and of their collecting ducts could possibly have come about in the following way. The primordial prostatic tubules may have been derived from a region of the urogenital sinus that is originally insensitive to oestrogenic stimulation. Sensitivity in this region and in the collecting ducts may have been attained later either by a change in the character of the original epithelium or by the replacement of that epithelium by more caudally disposed sensitive cells. On the other hand the lack of response of the glands might be due to the later specialization of cells which primarily arose from oestrogen-sensitive sinus epithelium. In favour of this view is the fact that in the rhesus monkey the urethral openings of the prostatic glands, which presumably give the topographical relations of the embryonic prostatic tubules, are in fact placed almost entirely in the uppermost part of the region of the urethra which responds to oestrone (Zuckerman, 1936*a*). Additional support for the view that differential specialization during development may occur in the prostatic buds is the fact that the epithelium of the acini of Cowper's glands in the rhesus monkey also fails to respond to oestrone. Cowper's glands are undoubtedly developed from a region of the sinus which is sensitive to such stimulation. The alternative to the view that the epithelium of these bulbo-urethral glands becomes insensitive to oestrogenic substances during embryogenesis is to assume that the sensitivity of the sinus (and of some of the tubular structures which open into it) is a characteristic acquired after the glands to which the sinus gives rise have budded. Such a view seems less likely than the one which implies that glandular organs derived from the urogenital sinus become specialized after they have budded.

SUMMARY

1. Two immature castrated male rhesus monkeys were injected daily with oestrone for a period of a year. The prostates of both animals were greatly increased in size. The epithelium of the true prostatic glands was unchanged and hyperplasia and metaplasia had taken place only in the epithelium of the urethra, the vagina masculina, the terminal parts of the ejaculatory ducts, and the collecting ducts of the prostate.

2. The nature of the prostatic growth induced by oestrone is analysed, and is shown to consist mainly in growth of the utricular bed—the region intervening between the urethra and the glandular area.

3. The occurrence of urethral diverticula is described and their presence is discussed in relation to the epithelial changes that occur under the influence of oestrone in structures that open into the urogenital sinus (urethra).

I am deeply indebted to Miss C. Sandys for the help she gave in measuring the transverse sections of the prostates discussed in this paper. I wish also to express my best thanks to Prof. W. E. Le Gros Clark for his stimulating interest in this work. The oestrone was very kindly provided by the Organon Company. The monkeys were bought with the aid of a grant from the Medical Research Council.

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EXPLANATION OF PLATES

Plate I

Fig. 1. Transverse section of mid-utricular level of prostate of normal rhesus monkey (O.M. 39). The glandular area has been outlined. Adventitious tissue ventral to the urethra is not clearly demarcated from the muscle. ×6.

Fig. 2. Transverse section of comparable level of prostate of monkey of similar age (O.M. 115) that had been injected daily for 368 days with 100γ of oestrone. The dark areas surrounding the urethra and within the utricular bed (area between urethra and enclosed glandular area) are collections of lymphocytes. ×6.

Fig. 3. Large urethral diverticulum intervening between urethra on one hand and ejaculatory ducts and uterus masculinus on the other. Marmoset (M.M. 71). ×32.

Fig. 4. The figure shows the opening of a urethral diverticulum into the urethra of a marmoset (M.M. 69). ×32.

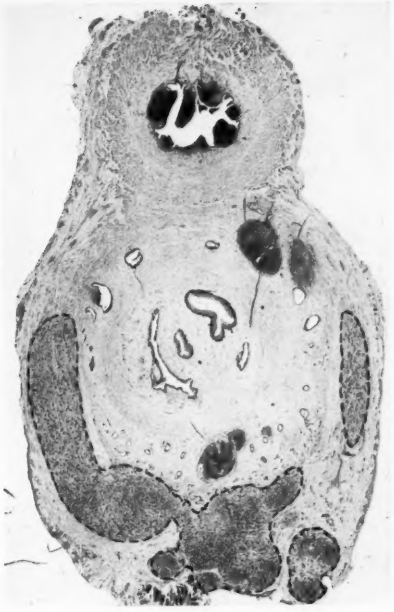
PLATE II

Fig. 5. The horseshoe bar represents the conjoined terminal parts of the ejaculatory ducts of O.M. 115. The structure within the bar is the vagina masculina, and the plates of cells lying outside it are the walls of collecting ducts of the prostatic glands. The dorsal wall of the urethra is thickened. The ventral wall is normal except for the presence of an unusually large collection of lymphocytes. ×17·3.

Fig. 6. Glands in the cranial part of the prostate of O.M. 115. They are normal in appearance. ×93.

Fig. 7. Normal glands in the body of the prostate of O.M. 115. ×93.

Fig. 1.



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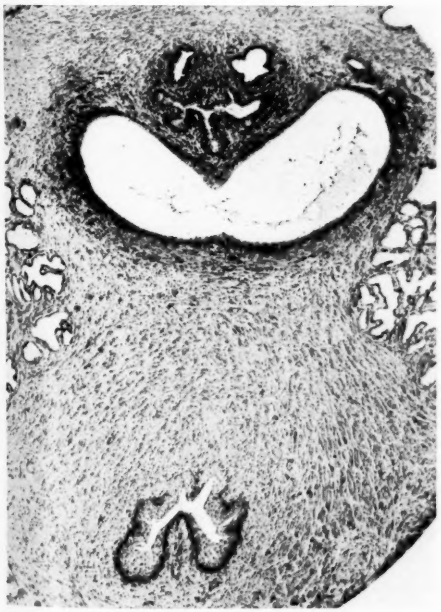

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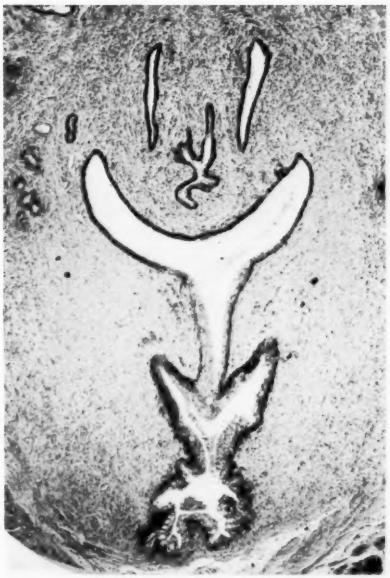

Fig. 4.

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Fig. 5.

Fig. 6.

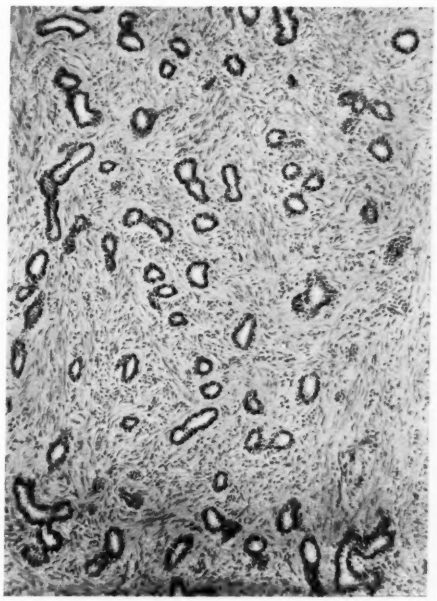

Fig. 7.

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THE EFFECTS OF MALE HORMONE ON A MATURE CASTRATED RHESUS MONKEY

By S. ZUCKERMAN and A. S. PARKES

From the Department of Human Anatomy, Oxford

The only available data regarding the effects of male hormone on the reproductive organs of male monkeys applies to immature specimens (Zuckerman & Parkes, 1936). The following observations were accordingly made on a mature male rhesus monkey (*Macaca mulatta*), O.M. 20.

The animal was castrated on 24 July 1935. On that day it weighed 6·72 kg. Eleven months later (16 June 1936) the upper half of its left seminal vesicle was removed; it was difficult to find the organ because of its very small size. On that day the monkey weighed 8·5 kg. Between 12 September 1936 and 11 December 1936—a period of 91 days—the animal was given a total of 242·5 mg. of testosterone propionate. At first 5 mg. were given on alternate days, and later 17·5 mg. were given in a single injection once weekly.

The animal's weight increased from 8·3 to 9 kg. during the course of injections. The animal itself became fiercer and more dominant. Flushing of the "sexual skin" such as occurs in normal mature male rhesus monkeys (Zuckerman, 1937*a*) slowly developed, but even at the end of the period of injections the skin was not as bright as it sometimes is in uncastrated macaques. The external genitalia were not appreciably larger at the end than they were at the start of the experiment. No changes occurred in the animal's pelage.

The internal reproductive organs at autopsy were as large as in a normal mature rhesus monkey. The specimen was peculiar in so far as the vagina masculina extended through the prostate in the form of a long narrow duct (lined by a single layer of cuboidal epithelium with somewhat vesicular nuclei) which ended in a knob containing the remains of the duct. The terminal part of the lumen lies in an oedematous fibrous stroma surrounded by a thick muscular wall.

The prostate. Pl. I, figs. 1 and 2 show the prostatic glands of a normal mature rhesus (O.M. 149) and of the experimental animal (O.M. 20) respectively. There is no difference in their histological structure. The glands in both are lined with actively secreting cylindrical epithelium of medium height. Pl. I, figs. 3 and 4 show the glands in the cranial lobes of the prostate ("coagulating gland", van Wagenen, 1936). They are more widely dilated than the more caudally disposed prostatic glands, and are secreting more heavily. They are obviously very responsive to male hormone and the fact that they do not

respond to oestrogenic stimulation (Zuckerman, 1937*b*) suggests strongly that they cannot be homologous with the so-called "coagulating glands" of small rodents.

The "utricular bed" (Zuckerman, 1937*b*, 1938) is essentially the same in the normal and experimental animals, and there is no evidence of epithelial growth in either the vagina masculina or the ejaculatory ducts. The former is lined in both specimens by two to three layers of low irregular cuboidal cells. The epithelium of the prostatic collecting ducts is also normal in the experimental animal, and is composed of from two to three layers of cells with relatively large nuclei.

The epithelia of the ampullae of the vasa deferentia and the ducts of the seminal vesicles of the normal and experimental animals are identical. Pl. II, figs. 5 and 6 show transverse sections of the left seminal vesicle before and after the injection of testosterone respectively. The great increase in size that resulted from the injections is immediately obvious. The two ducts lying below the seminal vesicle in fig. 5 are transverse sections of the left vas deferens. The right vas deferens does not appear in fig. 6.

Pl. II, figs. 7 and 8 are transverse sections of single processes of the seminal vesicles of the castrate control and castrate injected specimens. The epithelium of the injected specimen is much more extensively folded than the castrate. The absolute amount of muscle is not much increased in the injected specimen, and relatively there is less muscle. High power views of the epithelium in these two specimens are shown in Pl. II, figs. 9 and 10. In the castrate control the epithelium is low columnar with relatively large nuclei. There is no secretion. The stromal elements are much increased, the cells having ovoid nuclei. Patches of subepithelial degeneration are not uncommon. Similar areas of degeneration occur in the involuted endometrium of monkeys during lactation (Zuckerman, 1931). In the injected specimen there is relatively little stroma. The epithelial columns are formed of parallel rows of tall columnar cells set base to base and separated from each other by a very thin layer of stroma. The individual cells are enormously increased in size. The nuclei are disposed towards the middle of the cells and the cytoplasm is finely granular and very eosinophilic.

Like the prostate and seminal vesicle, the Cowper's glands of the injected specimen are as large, and in as active a phase of secretion, as in normal mature animals. The urethral epithelium in the injected animal is also as in normal animals.

These observations show that male hormone is able to maintain the accessory reproductive organs of a mature castrated monkey in a fully functional condition. We have referred elsewhere (Zuckerman & Parkes, 1936) to a mature male hamadryas baboon (*Papio hamadryas*) whose pelage changed from the male to the female type as a result of castration. This animal has also been treated with testosterone propionate, and its fur is gradually changing from the green-brown of the female to the grey of the male. The

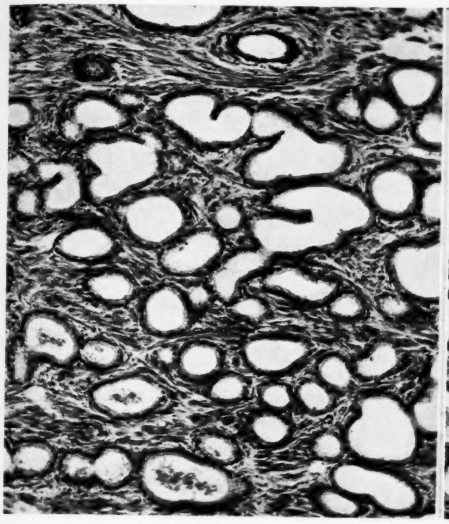

Fig. 1.

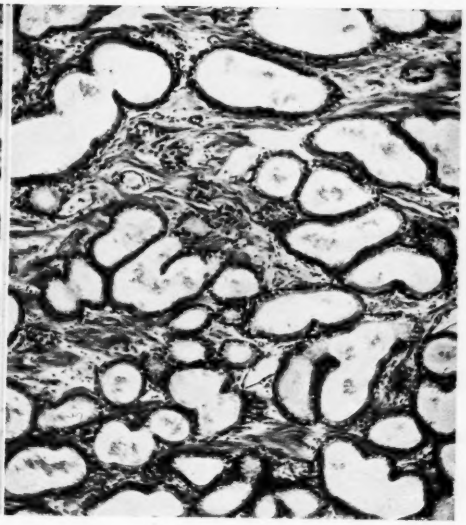

Fig. 2.

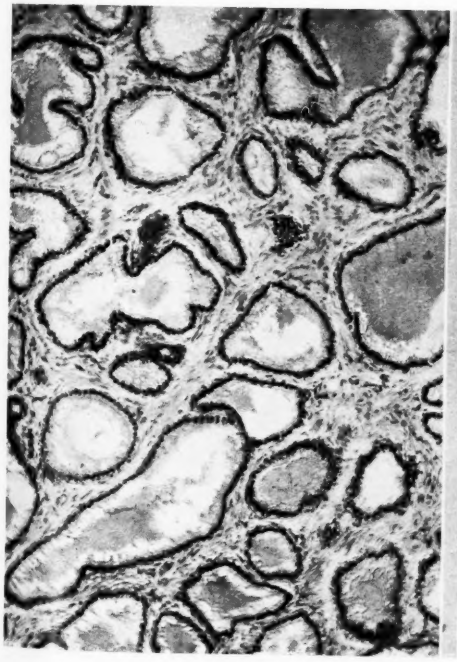

Fig. 3.

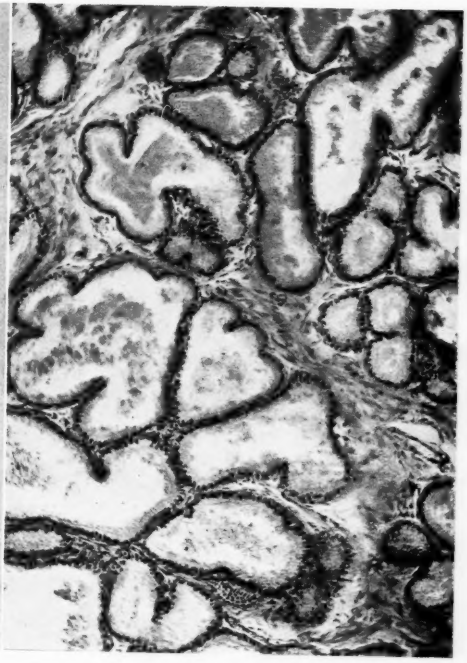

Fig. 4.

ZUCKERMAN AND PARKES—A MATURE CASTRATED RHESUS MONKEY

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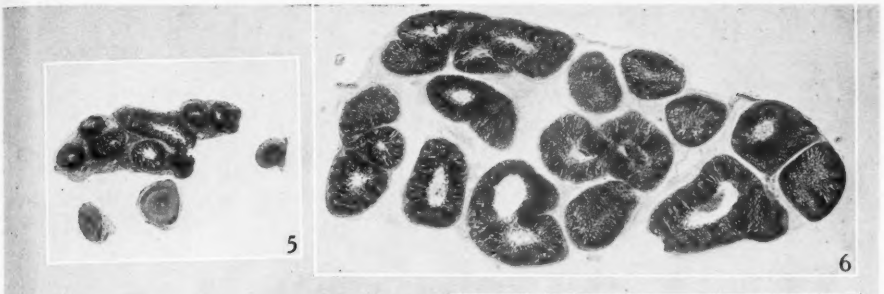

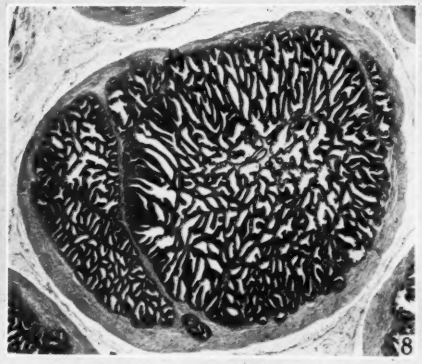

ZUCKERMAN AND PARKES—A MATURE CASTRATED RHESUS MONKEY

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characteristic cape of hair that distinguishes the adult males of this species is also returning. The animal has also increased considerably in weight during the course of injections.

SUMMARY

Male hormone (testosterone propionate prepared by partial synthesis) restored the atrophied accessory reproductive organs of a castrated mature male rhesus monkey to the normal size and functional condition. The animal also increased in weight during the course of the injections.

The testosterone propionate was kindly provided by Dr Miescher of the Ciba Company. The monkeys were bought with the aid of a grant from the Medical Research Council to S. Z. The photomicrographs are by Mr W. Chesterman.

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EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. Middle prostatic glands of normal mature rhesus monkey (O.M. 149). ×93.

Fig. 2. Comparable region in injected castrated monkey (O.M. 20). ×93.

Fig. 3. Upper prostatic glands of normal rhesus. ×93.

Fig. 4. Comparable region in experimental specimen. ×93.

PLATE II

Fig. 5. Upper part of left seminal vesicle of castrated rhesus monkey. ×3.

Fig. 6. Lower part of same seminal vesicle after treatment with male hormone. ×3.

Fig. 7. A single process of seminal vesicle in castrate control. ×16·7.

Fig. 8. Corresponding section after injection of male hormone. ×16·7.

Fig. 9. Epithelium of seminal vesicle of castrate control. ×280.

Fig. 10. Epithelium of seminal vesicle of castrated monkey after injections of male hormone. ×280.

AN OUTLINE OF ELLIOT SMITH'S CONTRIBUTIONS TO NEUROLOGY

By H. H. WOOLLARD

INTRODUCTION

Elliot Smith began his anatomical studies with an investigation of the monotreme and marsupial brain. The collection, preparation and examination of the considerable mass of material he used occupied about five years, and the results of this first important enquiry are contained in some twenty papers.

The work began when, as he himself remarks, he had only that superficial knowledge of anatomy the ordinary medical student possesses but he was fortunate in his teachers and friends (J. T. Wilson, J. P. Hill and C. J. Martin). The field into which he plunged was already crowded with distinguished names. Zuckerkandl, Ziehen, Koelliker, Edinger, and Broca on the Continent; Owen, Flower and Symington, and still earlier Alexander Hill in this country; Burt Wilder, Herrick and Osborne in America were devoting themselves to the problems of comparative neurology and writing on the cerebral commissures, yet nothing is more astonishing than the vigour and certainty with which Elliot Smith's early papers are written.

At the outset he had to pick his way amidst a mass of names and terms which many writers were using with different meanings and implications, and further he had to assess an unusual accumulation of conflicting statements and opinions. More especially at this time the assertion that the corpus callosum was present in the marsupial brain had gained considerable currency. Elliot Smith grasped, as we can now see, with unusual sagacity that in the study of the commissures lay the road to the understanding of the morphology of the cerebral cortex.

The papers he wrote around and about the commissural problem created our present knowledge of the morphology of the brain. The broad conclusions that are woven into any account of the evolution of the brain or mind, knowingly or unknowingly, spring from this work of Elliot Smith. Indeed, the first paper he wrote on this matter and which is reprinted in the *Journal of Anatomy*, vol. LXXI, p. 528, 1937, constitutes one of the most important contributions to the morphology of the brain that has ever been made. Written and published soon after graduation in his early twenties, it established once and for all the proof of his genius for research.

The papers which are concerned with the cerebral commissures taken together bear upon three logically related themes: the organization and interrelationship of those parts of the brain connected with olfaction; the proof that the commissures agreed with and supported the cerebral organization

based upon the study of the olfactory centres; the demonstration that the organization so determined could be applied to the more complex mammalian brains as well as to the more primitive non-mammalian brains.

Much that we accept at the moment as belonging to the common stock of knowledge was gained by Elliot Smith's vigorous statement of the results of these investigations. That the olfactory mucous membrane was connected by the olfactory nerves to the olfactory bulb was almost the only statement which passed without challenge. Otherwise Elliot Smith had to contend for his own observations and interpretations, and, while avoiding the errors of others, he had of necessity to explain how such had arisen.

He used all the advances that had been made in neuro-anatomical technique—the Weigert-Pal, the Golgi and the Marchi methods—and an abundance of fresh material was at his disposal.

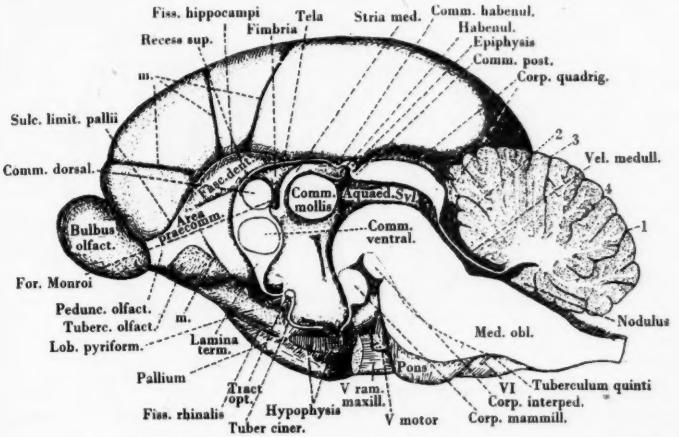

Fig. 1. The surface of the brain of *Ornithorhynchus* exposed by a mesial sagittal section. Magnified almost 4 diameters. *comm. dors.* hippocampal commissure; *fasc. dent.* fascia dentata; *comm. vent.* anterior commissure.

THE SECONDARY OLFACTORY CENTRES

Some fibres from the olfactory bulb pass into the anterior commissure, and others by various radiations or striae run to different olfactory centres. By the lateral stria—the lateral olfactory tract—fibres can be traced to the pyriform lobe. This tract separates the pyriform area from the lateral margin of another olfactory lobe which receives a middle tract of fibres from the olfactory bulb—the tuberculum olfactorium. The latter sits like a cap beneath the ventral extremity of the striatum. Still a third olfactory tract passes centrally from the olfactory bulb, but this climbs mesially and comes into an area that borders upon the lamina terminalis and was called at first by Elliot Smith the

area precommissuralis and later the paraterminal body.* These three areas present a general similarity in their cortical structure, and from this and from their relation to the distribution of olfactory bulbar fibres Elliot Smith inferred a general similarity in their position and function in the hierarchy of the olfactory system. He did not stress this too much, for he pointed out that the pyriform lobe cannot be purely olfactory since it persists in the anosmatic Cetaceae for instance, while the tuberculum olfactorium completely vanishes with the loss of the function of smell. Moreover the area precommissuralis receives fibres from the cortical part of the tuberculum olfactorium and provides a pathway for the transmission of fibres either directly or indirectly to the hippocampus. In recognizing these, despite their differences, as belonging to the level of secondary olfactory centres, Elliot Smith paved the way for a proper comprehension of the olfactory brain.

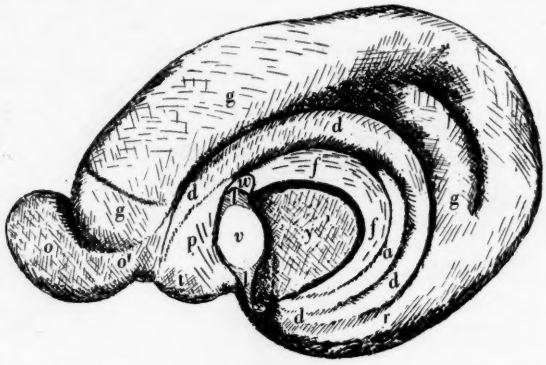

Fig. 2. Mesial surface of the cerebral hemisphere of a marsupial. *d*, *d*, *d*, fascia dentata; *w*, hippocampal commissure; *f*, *f*, fimbria or fornix; *o*, *o'*, olfactory bulb and peduncle; *p*, paraterminal body.

THE TERTIARY CENTRES

Beyond these secondary centres there lies the hippocampus which represents another and higher level of organization. In the monotremes and marsupials Elliot Smith observed that the hippocampus varied in extent. It might extend dorsally in front of the lamina terminalis—the precommissural portion, then pass back above the ventricle—the supra-commissural portion, and perhaps finally arch downwards and forwards—the post-commissural portion—thereby almost encircling the hilus of the brain. The term "limbic lobe" had been applied to those parts of the brain which embraced the area of commissural crossings. Like the term "rhinencephalon" it had come to include parts other than those which belonged primarily to the olfactory

* The same region is called, especially by American neurologists, the septal area.

system. The realization that the hippocampus extends dorsally over the commissures enabled Elliot Smith to insist on the proper description and allocation of the parts embracing the hilus of the brain. The limbic lobe is composed really of three parts, a dorsal hippocampus extending posteriorly and ventrally, a ventral pyriform lobe, and an anterior intermediate area—the area precommissuralis or septal area. The hippocampus is covered everywhere on its ventricular surface by the white, medullated fibres of the alveus and bordered by the fornix which comprises all fibres making up the alveus.

Having defined what portions of the brain belong to the olfactory system, the way was clear for the understanding of the commissures which would settle for all time what Edinger called the fundamental neurological pattern.

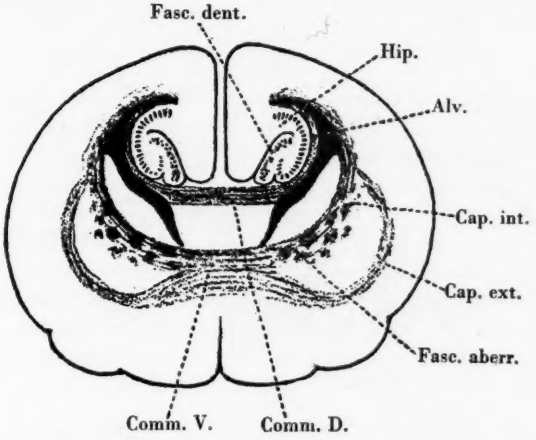

Fig. 3. Shows in a Diprotodont marsupial brain the passage of neopallial fibres—fasc. aberrans—via the internal capsule. × 1½.

THE VENTRAL COMMISSURE

The ventral commissure lies below the lateral ventricle. It passes between the two olfactory bulbs beginning and ending in the deepest stratum of its cells. It is not, however, restricted to olfactory centres but it carries always some pallial (neopallial) fibres. The term pallium was used to denote cerebral cortex in general, but as the need to distinguish those parts of the cerebral cortex which were olfactory in function from those which were non-olfactory in function and more recently evolved was pressing, Elliot Smith introduced for these the term neopallium, which was later modified to neocortex.

The marsupials, like all mammals, possess a neopallium or neocortex, and in these Elliot Smith observed that the neopallial fibres in the anterior commissure might pass either in the external capsule—i.e. external to the corpus

striatum, or through the corpus striatum—i.e. in the position of the internal capsule. It is customary to classify the marsupials into two great groups, the Polyprotodontia and the Diprotodontia. Elliot Smith discovered that it was only in the Diprotodontia that neopallial fibres passed through the internal capsule. This fasciculus, which pierces the striatum, he called the fasciculus aberrans, and in addition to perceiving its remarkable zoological distribution, he pointed out that the continued growth of the pallium (neopallium) would impose upon the internal capsular route such demands that these could only be met by a profound structural disturbance of the brain. It is difficult to imagine how the evolution of the brain could have continued if a new route had not been developed. The use of the dorsal supraventricular route—the third way of arranging the course of neopallial fibres—is the distinctive feature of the eutherian brain.

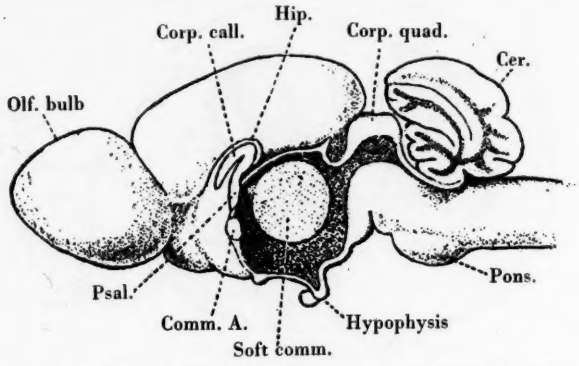

Fig. 4. Median section of brain of a primitive eutherian mammal.

THE DORSAL OR HIPPOCAMPAL COMMISSURE AND CORPUS CALLOSUM

Between the hippocampal areas of the two sides there pass in the alveus and fornix the fibres of the dorsal or hippocampal commissure. Remembering that the hilus of the brain may be encircled above, behind and below by the hippocampus, it will happen that the dorsal commissural fibres of the supra-commissural part of the hippocampus and ventral commissural fibres of the caudal and inferior hippocampal portions will be angulated upon each other posteriorly. This Elliot Smith was the first to appreciate, and he saw that in the marsupial brain the hippocampal commissure consisted of dorsal and ventral laminae joined together in a caudal angle which could be called a splenium. *The corpus callosum replaces the dorsal lamina of the hippocampal commissure.* The pallial fibres of this corpus callosum might have passed above the dorsal moiety of the hippocampus or through it or below it. They actually pass

through the fibres of the dorsal part of the hippocampus, and so it comes about that the corpus callosum runs in the alveus, and the dorsal part of the hippocampus is spread out upon its dorsal surface as the stratum indusium. The corpus callosum, since it replaces the dorsal part of the hippocampal commissure, necessarily occupies and distends the lamina terminalis, for both the anterior and hippocampal commissures traverse this terminal band. The thin precommissural investment of the commissures derived from this lamina terminalis Elliot Smith called the commissural bed.

When the development of the corpus callosum was made clear the fundamental pattern of the mammalian brain was apparent. The terms neopallium (pallium in the early papers) and rhinencephalon had now gained a significance and precision that previously did not attach to them.

OLFACTORY FIBRE PATHWAYS

The fibre pathways of the olfactory brain had to be aligned in accordance with these new ideas of the cerebrum and its commissures. It has already been mentioned in outline how the fibres pass from the olfactory bulb to the secondary centres. The remaining system is known as the fornix, and Elliot Smith defined this as meaning all the fibres which are in some way connected with the alveus of the hippocampus.

In the fornix there are, of course, as has already been mentioned, the commissural fibres (the psalterium or dorsal commissure of human anatomy). The fornix is always continued into an anterior column whose fibres will have come in the metatherian brain from all three parts of the hippocampus. Some of the fibres of the anterior column can, of course, be traced to the corpus mammillare, but others pass in front of the ventral commissure to the base of the brain, forming a hippocampal basal bundle. On the surface of the area precommissuralis (paraterminal body) fibres pass upwards into the hippocampus. When the corpus callosum develops, these fibres will pass round the front end of this commissure, but others will pass through the septum lucidum and perforate the corpus callosum and so reach the stratum indusium—the remains of the dorsal hippocampus. From this same precommissural area fibres can enter the fornix and form the stria medullaris and so reach the habenular region, and also from this same region fibres pass to the hypothalamus. Fibres may pass directly from the tuberculum olfactorium and area precommissuralis to the fascia dentata.

THE GROWTH OF THE CORPUS CALLOSUM

The third step in this work was to study the changes brought about by the development of the corpus callosum in the eutherian brain. It has now become a commonplace that the stratum indusium and the medial and lateral longitudinal bundles, together with the perforating fibres, represent the dorsal or

supracommissural portions of the fascia dentata and hippocampus and their connexions. Just as the corpus callosum has reduced the dorsal hippocampus to this vestigial sheet, so it has by slow degrees drawn out the upper part of the lamina terminalis and the area precommissuralis into the septum lucidum.

The illustrations showing the gradual phylogenetic expansion of the commissures, the corresponding expansion of the commissural bed, the elongation of the walls of the area precommissuralis into the angle between the corpus callosum and the dorsal commissure, and finally the separation of these from the rest of the medial surface of the brain by the secondary fusion of rostrum of the corpus callosum with the ventral commissure are well known because they have been so frequently copied.

Less attention has been paid to the modifications of the lower olfactory centres. The pyriform lobe in its anterior half has become atrophic, and what is now called the pyriform lobe really represents only its posterior half (the natiform eminence). This posterior half runs backwards so that it lies to the outer side of and overlaps the ventral extremity of the hippocampus. Its demarcation involved the correct appreciation of the endorhinal and ectorhinal fissures. It is the ectorhinal which persists as the rhinal fissure in human anatomy and becomes confluent with the collateral fissure.

The atrophic front portion of the pyriform lobe lies in the Sylvian vallecula and adjoins the lateral margin of the tuberculum olfactorium. The lateral olfactory radiation occupies the interval where these two areas adjoin. The olfactory radiations circumscribe in the human brain an atrophic area, the locus perforatus anticus, wherein the tuberculum olfactorium formerly existed. It is the lateral portion of this perforated area in a microsmatic eutherian brain which contains in its front part the remains of the specialized cortex of the tuberculum olfactorium. Behind the latter (which formed a cap to the corpus striatum) is an area which has been called the area depressa. This is the real perforated region and represents a part of the precommissural area (the gyrus infracallosus of Zuckerkandl), and the diagonal band of Broca or the pedicle of the corpus callosum is a surviving link between this atrophied area and the area precommissuralis. As already alluded to, it is upon the lateral side of this atrophic olfactory trigone that the degenerated front end of the pyriform area is situated. The latter marks the position of the amygdaloid nucleus, the means by which olfactory connexions are established between the hippocampus and the corpus striatum. Elliot Smith drew attention to the fact that atrophy of the anterior half of the pyriform lobe might result in exposure of the amygdaloid nucleus upon the surface of the brain and that the gyrus lunaris of Retzius was really this exposed part of the amygdaloid nucleus.

SHORT SUMMARY OF RESULTS

It is instructive to compare the three parts of the limbic lobe in *Platypus* and in man, and Elliot Smith drew up the following table:

	Ornithorhynchus	*Homo*
1.	Dorsal limb of limbic lobe = hippocampus, fascia dentata, fimbria	Gyrus geniculi, gyrus supracallosus (st. Lancisii) fascia dentata; hippocampus; fimbria, fornix longus
2.	Ventral limb of limbic lobe	Gyrus uncinatus (excluding subiculum): cornu ammonis
	Pyriform lobe	Radix lateralis (bulbi olf.) the floor of the vallecula joining the latter to the gyrus uncinatus
3.	Pars intermedia = thickened lamina terminalis; (commissural bed)	Septum pellucidum; gyrus subcallosus; gyrus infracallosus
	Precommissural area; tuberculum olfactorium	Locus perforatus anticus

These studies of the fibre connexions and cellular pattern of the different parts of the olfactory system, the realization of the succession in time and place of the cerebral commissures, and the comparison of primitive and more complex, and of microsmatic and macrosmatic brains, enabled him to comprehend the fundamental pattern of the vertebrate brain.

The olfactory bulb with its ventral commissure, together with the olfactory connexions as far as the anatomical and physiological level of the pyriform lobe, constituted a basal pallium, this being the level of organization of the brains of fishes and Amphibia. This basal pallium is the paleopallium of later writers. In the reptiles and primitive mammals, the fascia dentata, the hippocampus with the alveus, the fimbria and the dorsal commissure form an addition to the paleopallium. This further advance in cerebral development is greatest in the primitive macrosmatic Proto- and Metatheria. Elliot Smith gave the name "marginal pallium" to this new level in the cerebral hierarchy. Later writers have called it the archipallium. These two cerebral divisions—basal pallium and marginal pallium—correspond nearly to Turner's rhinencephalon. Foreshadowed in reptiles, still slender in the primitive mammals, but eventually in the primates dwarfing all else in the nervous system, is the neopallium. The morphology and meaning of the neopallium formed a problem that hereafter was rarely absent from the mind of Elliot Smith. The passage which follows is taken from a paper on the Brain of Edentates which was published in 1902. It shows that at that early date Elliot Smith realized clearly the implications of his discoveries in cerebral morphology.

"In all vertebrates the incoming olfactory nerves are inserted into an olfactory bulb, which is connected by a peduncle with the cerebral hemisphere, which at first consists of little else than a small, simple, undifferentiated basal ganglion. Even in the Dipnoi and Amphibia we can recognize a differentiation of the mesial wall of the primitive hemisphere into the precommissural body and the forerunner of the hippocampus, while on the basal and lateral aspects the undifferentiated primordial tuberculum olfactorium and lobus pyriformis

may be localized. In the Sauropsida the primordial hippocampus and pyriform lobe advance a further stage in the process of differentiation, although they are still far removed from the final stage of elaboration which we find in even the lowest mammal.

"In the mammal the development of the pallium (probably by differentiation from the basal ganglion) provides a cortical area with a free scope for expansion, in connexion with which paths from all the lower sensory centres become established. Thus the primitive pallium becomes a field in which the retina, the sensory lining of the internal ear, and all the tactile surfaces of the body become represented, so that the impulses of these various senses may meet and mutually react the one upon the rest. Thus the primitive pallium is essentially 'a sensory area', but it is at the same time a 'projection area'. For all of these centres may exercise a combined influence upon the pallial nerve cells, from which efferent impulses proceed to the lower executive centres."

The application of the precise anatomical knowledge he had acquired about the vertebrate brain and the effect of his physiological interpretations are the foundation of his magnificent monograph on the collection of brains in the College of Surgeons. In particular in this "Physiological Series of Comparative Anatomy" he described the brains of reptiles and mammals. There are comments on about 700 specimens and to illustrate these over 200 figures were prepared. The comments are often abundant, at times swelling into a general account of the evolution of the nervous system or again linking some feature such as a fissure with its whole mammalian history. In spite of the fact that there are now readily available several fine monographs on comparative neurology it can be asserted that none of these surpasses in stimulating interest this catalogue made for the College of Surgeons by Elliot Smith. His ability, knowledge and interest in the subject transformed a catalogue into a great monograph of comparative neurology.

CEREBELLUM

The cerebellum of all organs exhibits the greatest diversity in its external form, though none is so uniform in its internal structure. It would seem that an organ having an inward uniformity is able to indulge in an external diversity. Human anatomists have revelled in the opportunity of attaching names to the apparent irregularities of the cerebellum.

It is true that Henle and Schwalbe had mentioned that the cerebellum was composed of three main lobes, but this rested only on the most superficial basis and no one paid any attention to the statement. It was in 1899 in his monograph on the Brain of the Edentates that Elliot Smith proposed his novel conception of the constitution of the cerebellum. He pursued the subject vigorously and quickly unravelled the seeming complexity of its outward form. His ideas are summarized in a scheme which has been used by everybody who has since seriously considered the cerebellum. His success in solving so quickly a problem so baffling was due to his insight into the real meaning of morphology.

He immediately proceeded to the study of the cerebellum in *Perameles* and *Notoryctes*, and began by showing that in their cerebellar anatomy these were the most primitive of all mammals. He turned also to the ontogeny of the organ and made sure of the order in which the fundamental parts of the cerebellum made their appearance. At the end of the third month in the human foetus the fissura prima appears; and at the end of the fourth month the prae- and post-pyramidal fissures. The cerebellum, he concludes, consists of the flocculi and three chief lobes—the anterior, middle and posterior lobes, the middle lobe alone dividing into a pars dorsalis and a pars ventralis. In his triple division of the cerebellum he relied almost entirely upon the comparison of external form.

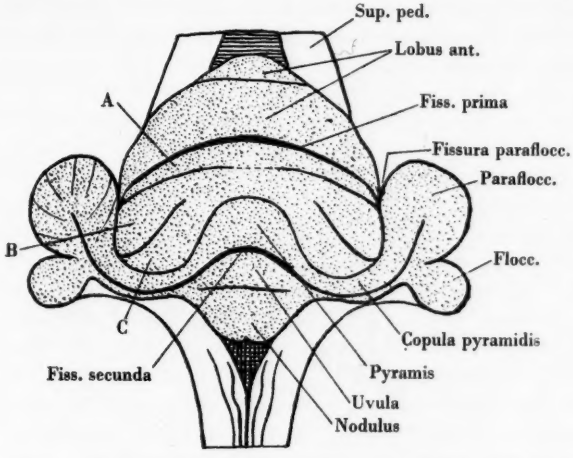

Fig. 5. Diagram to represent the fundamental subdivisions of the cerebellum spread out in one plane.

In later times Bolk attempted a broad survey of the same morphological problem. Neither his twofold subdivision of the cerebellum nor any of his other ideas about the cerebellum have become established. Ingvar still later issued a monograph based upon comparative anatomy, fibre connexions, congenital defects in growth, and experimental enquiry.

The result of this last analysis on a considerable scale is the complete vindication of Elliot Smith's fundamental plan of the cerebellum. There is only one discrepancy and it is not a large one. Elliot Smith allocated the lobulus pyramidalis to the middle lobe of the cerebellum, while Ingvar put it in the lobus posticus. On considering the reasons for this difference in allocation it is clear Elliot Smith was quite aware of a copula pyramidalis which joined the lobus pyramidalis and the flocculus, but on the whole, in a region of great variability and much change in form due to slight differences in the rate of growth, he considered that the uvula was more constantly joined to

the flocculus than the lobus pyramidalis. Ingvar states that the bird's cerebellum shows clearly the allocation of the pyramid to the lobus posticus and says that this allocation fits better the distribution of the spinocerebellar fibres.

The importance of a clear apprehension of the fundamental structure of the cerebellum is difficult to exaggerate. Not only is its comparative anatomy made lucid and informing and the human cerebellum fitted into the scheme, but the functions of the cerebellum have been entirely restated because of this successful morphological analysis. The cerebellum in the organization of the proprioceptive system occupies a leading position, but this is its earlier function which has relatively diminished with the increasing volume of the middle lobe. It is this lobe which has grown with the cerebrum. The effects of cerebellar defects in man are due to the loss of the influence provided by the cerebroponto-cerebellar system.

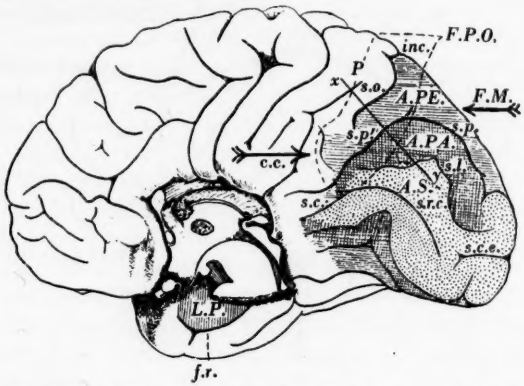

Fig. 6. The mesial aspect of the right cerebral hemisphere of the (practically) acallosal brain of an Egyptian fellah woman. *A.S.* area striata; *A.PA.* area parastriata; *A.PE.* area peristriata. The three dorsal limiting furrows of these areas: *s.l.*, *s.p.* and *s.o.* The other features explained in the text.

AREA STRIATA

Cunningham had made an analysis of the calcarine sulcus and distinguished an anterior from a posterior calcarine sulcus, regarding the former as the true calcarine and common property of all mammals. It is the anterior calcarine which produces the calcar avis and is the true calcarine sulcus in the sense that it produces this intraventricular eminence. In a further analysis of the sulci of the occipital region Cunningham disentangled the "Affenspalte" from the sulcus occipitalis transversus.

When the opportunity of examining a large number of brains (he examined over 400) came to Elliot Smith in Egypt, he devoted some attention to the occipital region and soon became aware of the close similarity in the fissural

pattern of the occipital region in these brains with that of the same region in the gorilla. He succeeded in reducing to order the apparent irregularity of their arrangement and making intelligible the terminology applied so variously by different authors. He determined the homologies of the "Affenspalte", explained the various forms it might assume, and concluded that it must be almost invariably present in the human brain.

In his analysis of the calcarine sulcus he showed how the anterior calcarine is really a limiting sulcus separating the visual area from the cortex of the cingular gyrus. The retrocalcarine is a shallow axial furrow partially infolding the visual area. In this it differs from the same furrow in the Cercopithecidae where the infolding is complete. In general in this region the form assumed in man as a consequence of brain growth is such that the occipital region retains more of the lemurine and common mammalian pattern than of the pithecoid specializations.

When it was established that the area striata and the visual area are coterminous, the meaning of the fissures in the occipital area also became clear. The examination of fresh brains displayed the sharpness of the striation of the visual area, and its boundaries could be defined precisely because it stopped so abruptly.

Owing to the great growth of adjacent neopallial areas the area striata gradually becomes pushed mesially until perhaps less than one-quarter of it remains lateral.

Since in man the infolding of the area striata along the sulcus calcarinus is only partial, much of the area striata is left on the surface in the cuneus and in the lingual gyrus. In the cuneus and in the lingual gyrus the limits of the area striata are indicated by superior and inferior limiting gyri. The axial folding of the calcarine sulcus need not be confined to a single furrow, and the infolding effect might be achieved by several shallow parallel sulci, and this happens occasionally.

Upon the lateral surface the axial furrow—lateral calcarine sulcus, or sulcus occipitalis superior—is bounded in front by the sulcus lunatus and posteriorly at the occipital pole it may bifurcate into dorsal and ventral limbs, these having between them the posterior extremity of the retrocalcarine sulcus of the mesial surface. Upper and lower limiting sulci, which along with the sulcus lunatus enclose the area striata of the lateral surface, are the sulcus occipitalis paramesialis and the sulcus occipitalis inferior. In this complex of sulci much variation occurs, and the sulcus lunatus may assume many different forms. For instance, it may be strongly curved, a mere vertical, transverse, oblique or horizontal slit, or may extend from one occipital margin to the other and may be simulated by other fissures, especially the sulcus occipitalis transversus from which it was first differentiated by Cunningham.

OTHER CORTICAL STUDIES

From the preceding statements it will be clear that Elliot Smith saw with remarkable prescience the functions of the neopallium, the evolutionary history of which he had so convincingly unravelled.

One of the earliest fruits of his study of the neopallium was the discovery that the splenial sulcus found in all mammalian brains is identical with the precalcarine sulcus of the primate brain, and indeed is really a limiting sulcus separating the visual area from the cortex of the cingular gyrus. His appreciation of the fact that the area striata is exactly coterminous with the extent of the visual receptive cortex enabled him to reduce to an intelligible and reasonable pattern the sulci of the mesial and lateral occipital area.

The subsidence of the olfactory stream, the growing importance of the visual mechanism in brains that had evolved beyond the level of the primitive mammal, and the obvious pre-eminence of vision as the epicritic sense were of course immediately apprehended by him. In later life he elaborated in papers, books and lectures the part played by vision in the evolution of the Primates, and from his laboratory came many papers by his students which disclosed more and more anatomical features justifying and substantiating the dominating evolutionary role of vision.

In studying the history and meaning of these sulci of the Primate brain it was of course necessary to decide the sort of fundamental pattern from which this brain must have started. The problem clearly stated was solved by the same principles as used before, viz. the determination of the pattern in the appropriate primitive forms. Incidentally this search led to a very careful consideration of the affinities of the lemurs, for their similarity to lower mammalian types had led some to deny Primate rank to them. For several reasons—the confluence of the original calcarine and retrocalcarine sulci, the beginnings of a true Sylvan fissure and a true central sulcus, and the comparative size of the brain—Elliot Smith insisted upon their Primate rank. This insistence is accompanied by a statement of the anatomical features wherein they fall below the level of higher Primates—their high degree of osmatism, the uncovered cerebellum, the small occipital region, and the absence of posterior horn of the ventricle. *Tarsius*, however, does possess a posterior horn and it is observed that it presents at once the most generalized and the most pithecoid brain of all primitive Primates.

ANATOMICAL DIFFERENTIATION OF THE CORTICAL GREY MATTER

Like every other neurologist, Elliot Smith was confronted with the problem of cerebral localization. He had applied to the area striata three conceptions—that fissures may be axial and so increase a functional area, or may be limiting and so bound a functional area, and that a particular functional area may be

distinguished by an easily recognizable structure. Audaciously he attempted by means of the fissural pattern and the results of examining the cortex with a hand lens to produce a map of the whole cerebral surface. He succeeded in this, and produced a chart not very dissimilar from that of Campbell and of Brodmann. It is true that the laborious histological method has been pursued with a diligence and minuteness that is almost incredible, that cortical maps have by this method become so detailed that they are almost incomprehensible, and that the simple, quick method of Elliot Smith has been forgotten. It is, however, not unlikely that the burden placed upon the histological method is beyond its capacity, that detailed maps produced in this way are, apart from the grosser features, quite meaningless, and that the subdivisions so produced have no relation to any other aspect of cerebral neuro-anatomy and no identifiable parallels in mental processes. Elliot Smith's simple, quick method might possibly give us a truer and more useful view of the pattern of the cerebral cortex.

THE MEANING OF FISSURES

It seems worth while emphasizing Elliot Smith's conception of the fissural pattern of the cerebrum. Clearly the cerebral cortex is unique in that it shows a greater relative increase in size than any other organ in the course of vertebrate evolution, and this increase in size has been in two dimensions only. It is an increase of area, and the increased area has been acquired by folding, most often where different functional areas adjoin. Sometimes the folds or sulci have occurred in the centre of a uniform functional area. The rhinal and hippocampal fissures are the oldest examples of these two types of fissures. The true or anterior calcarine separates the area of the cingular cortex from the area striata. The orbital or fronto-marginal, a fissure of the "generalized mammalian" brain, passes forwards and upwards from the rhinal fissure and separates the frontal from the motor area. The suprasylvian sulcus occurs between the cutaneous and the auditory area. The sulcus coronalis and sulcus lateralis separate the cutaneous sensory area from the motor area. The sulcus cinguli occurs where the subiculum joins the neopallium. Obviously (as Elliot Smith realized) the one real criterion that could be used to determine the homology of fissures and sulci would be the performance of this limiting function for similar functional areas in different brains.

ENDOCRANIAL CASTS

It would take overlong to give a detailed account of the many ways in which Elliot Smith used the knowledge of cerebral anatomy which had become his through these extensive researches. Some have already been alluded to, others can only be mentioned—endocranial casts of fossil mammals: the archaeoceti and the extinct lemurs, fossil man—Piltdown—Lloyd's skull,

Sinanthropus, the Taungs skull, the Rhodesian skull and others. In the interpretation of endocranial casts Elliot Smith's imitators have so often been guilty of exaggeration that it is hoped that the following quotation from the morphology of the brain in mammals will have a restraining effect:[1]

"It is commonly supposed that a mould of the brain case can give little information beyond indicating the shape of the brain. But in most mammals in which the pattern formed by the sulci is not very complex (and also even in some, especially carnivores, in which a very rich supply of sulci exists) a series of prominent ridges develop on the inner surface of the cranial wall so as to accurately map out the plan of the sulci. These ridges are especially well developed in the lemurs, excepting only the extinct species Globilemur, in which the pattern becomes blurred as in most apes."

CONCLUDING REMARKS

Observations on the evolution of the human brain furnish hints of rich fields awaiting investigation. His series begins with Petromyzon and includes Lepidosiren and Sphenodon. His account of the reptilian and primitive mammalian brain is especially interesting. Having established the homologies of the paraterminal and medial hippocampal areas in all vertebrate brains, it is in reptiles for the first time that a new region lying between the medial hippocampal and lateral pyriform territories is found. That impressions other than olfactory reach the pallium may now be true, and in doing so they excite a pallial growth in the region of the striopallial junction. This growth adds, on the one hand, a new impetus to the development of the striatum inducing the formation of the hypopallium, a special adaptation in reptiles, and, on the other, urges along the growth of the neopallium—a special adaptation in mammals. This hypothesis lights the way for many morphological studies waiting to be carried out.

The object of these brief comments has not been very much to insist upon discovery. The history of science shows that so-called discoveries usually depend on the contributions of many workers. But the history of science also shows that at times an abler mind handles a familiar problem and gets out of it so many fresh and novel ideas that the whole subject acquires a new interest and importance. Because we think Elliot Smith achieved this in neurology these notes have been compiled.

[1] On the morphology of the brain in mammalia, with special reference to that of the lemurs recent and extinct. *Trans. Linn. Soc. Lond.* Zool. (1903), vol. VIII, pp. 319–432. 2nd series.

TWO SKULLS WITH ABSENCE OF THE PREMAXILLA

By D. E. DERRY

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The condition exhibited by the two skulls to be described is uncommon and for this reason alone it is worthy to be recorded. But it has the further interest that it throws light on the remarkable embryological changes which take place in the development of this part of the face in the human embryo as compared with lower forms.

Skull No. 1. This skull was found by Mr G. Brunton in a grave of the 25th Dynasty *circ.* 700 B.C. while excavating a cemetery on the east bank of the Nile, about 30 miles south of Assiut.

The skull appears to be that of a woman and from the condition of the sutures and wearing of the teeth it seems probable that she was well past middle age. With the exception of the deformity to be described the skull is normal.

The malformation in question consists in a marked reduction in size of the hard palate and on examination this is found to be due to absence of the premaxillary part of the maxilla as well as the horizontal plates of the palatine bones. Nevertheless the lower margins of the nares and the nasal spine all of which have been believed to be derived from the embryonic fronto-nasal process are well developed. There are of course no incisor teeth, these being developed in the premaxillary element of the jaw. The most centrally placed teeth in this skull should be the canines, but only one of these, the right, is present and it is lying horizontally above the two bicuspids and first molar while its crown is visible just below the anterior nares. The white area seen in Pl. I, fig. 1 projecting across the middle line below the nasal spine is the tooth in question. The *left* canine has not developed, there being no sign of it in an X-ray photograph of the part, but there is an area of bone between the middle line and the socket for the first left bicuspid tooth which represents the site it should have occupied. A minute dental (? enamel) excrescence is present which may be the rudiment of the missing canine. It can be seen in Pl. I, fig. 1 lying just to the medial side of the socket for the left bicuspid.

Besides the abnormally placed canine tooth only four teeth remain in the jaw (Pl. I, fig. 2). On the *right* side the two bicuspid teeth and the first molar are still present. On the *left* side the second bicuspid is the only tooth remaining. The condition of the socket for the first left bicuspid tooth indicates that this tooth has only recently fallen out. The socket for the first molar is much absorbed and probably only stumps remained at the time of death. The second

and third molars have never developed on either side and there is no room for them. Perhaps their absence may be correlated with the non-development of the horizontal plates of the palatine bones.

The greatest antero-posterior length of the palate measured from a point on the posterior margin of the socket for the canine tooth to what may be considered as the posterior nasal spine is 26·5 mm. The greatest width measured between the inner margins of the sockets for the first molar teeth is 16·5 mm. From the photograph (Pl. I, fig 2) it can be seen that there is extensive (? pyorrhoeal) absorption of the bone round the roots of the second bicuspid and first molar teeth on the right side and this has proceeded so far that perforation of the floor of the nose at the site of the abscess cavities has taken place. Owing to the extreme smallness of the palate the teeth have failed to articulate properly with those of the mandible which is normally developed and has contained its full complement of teeth. The upper teeth have been caught *between* the teeth of the mandible (Pl. I, fig. 3) and their labial surfaces have been worn flat while the teeth themselves have been pushed upwards and inwards so that, as already said, their roots have come into communication with the floor of the nose. As might be expected, owing to absence of the premaxilla there is no incisive fossa. There is a minute foramen in the middle line immediately behind the abnormally placed canine which may have transmitted the naso-palatine nerves but it cannot be traced to the nose. Further back on the palate is a foramen which opens immediately on the nasal floor and this again may have given passage to nerves or blood vessels or both. Some idea of the appearance of this woman in life is obtained from the profile view (Pl. I, fig. 3).

Skull No. 2. The provenance of this skull is unknown. From the general appearance and condition of the bones it would seem to be relatively modern. Also it appears to have belonged to an adult woman. Like the case described above, the premaxilla is absent and there are therefore no incisor teeth. The sockets for all the other teeth are present although all of these except the left first molar have fallen out recently. As in Skull 1 the embryonic maxillary processes have grown across the middle line and formed the lower margins of the nares, but they have failed to meet as perfectly as in the first case described, so that the nasal spine is absent and its site is occupied by a depression (Pl. II, fig. 4). It is unfortunate that the teeth have been lost as it would have been of interest to have seen whether the canines were normal. In this type of malformation they are the teeth most likely to be affected lying as they do at the junction of the embryonic fronto-nasal and maxillary processes. Actually the sockets left by the canine teeth in this skull are small and shallow, little larger in fact than would be required for milk canines and it is evident that the roots did not penetrate deeply into the jaw. It is possible therefore that the permanent canines had never developed, as is the case on the left side in Skull 1.

The socket for the *right first bicuspid* shows that the tooth was decayed. There has been an abscess at the root which has perforated the alveolar wall.

Both it and the second bicuspid have had insufficient room between the canine and the first molar tooth and are irregularly arranged in consequence. On the *left* side all the sockets are normally arranged (Pl. I, fig. 5).

It was remarked in Skull 1 that the horizontal plates of the palatine bones had never been developed. In the skull under review they are normal and the hard palate is correspondingly longer and wider (cf. Pl. I, fig. 2). The antero-posterior length from a point in the middle line between the sockets for the canine teeth to the tip of the posterior nasal spine is 40·0 mm. The width between the second molar teeth is 30·5 mm. The difference between the antero-posterior diameters of the palate in the two skulls, 13·5 mm., is thus almost exactly that of the horizontal plates of the palatine bones, 14·0 mm., which are missing in Skull 1. Although there is no true incisive fossa, this being formed normally between the palatine processes of the maxillae behind and the premaxilla in front, yet the nerves and blood vessels have found their way through the suture between the palatine processes.

In marked contrast to Skull 1 in which the smallness of the palate did not allow of normal articulation between the upper and lower teeth, in this second skull although most of the teeth have fallen out it is possible to satisfy oneself that during life the teeth were normally opposed so far as the molars on the left side are concerned, and probably the left bicuspid. This is not so certain for the right side owing to asymmetry of the alveolar arch which has resulted in a crowding of the right upper bicuspid teeth and a tendency for the anterior part of the arch to be pushed inwards (Pl. I, fig. 1). While the molars *may* have articulated, the right upper and lower bicuspid teeth certainly did not.

The early disappearance in the human subject of all signs of the suture between the premaxilla and maxilla is a fact of great interest associated as it is with increase in the growth of the brain and recession of the face. Although the suture can be traced on the palate in young and even some adult skulls it has always disappeared on the face long before birth. In the anthropoid apes it persists for many years.

If a comparison be made of the associated parts in man and the apes the following remarkable differences will be found. The margins of the nares as seen in man are not represented in the apes. In these animals the corresponding parts are rounded and formed entirely by the premaxilla as this ascends towards the nasal bones. The medial part of each half of the premaxilla slopes steeply towards the nasal floor and passes on to the face without any interruption in the form of a narial margin such as exists in the human subject. Consequently there is no incisive crest and no nasal spine in the apes. Hitherto it has been assumed that in man the whole thickness of this part of the alveolus carrying the incisor teeth and forming the anterior part of the floor of the nose with the margins of the nares and the nasal spine is formed from the premaxilla; but the skulls described above, particularly Skull 1, show that perfect narial margins and a prominent nasal spine may be developed with complete absence of the premaxilla. It is plain therefore that these have developed from

the maxillary processes and are a new formation peculiar to man. Frazer, in his *Manual of Embryology*, p. 275, says: "The maxillary process...as already indicated forms the premaxillary alveolus and the lip, covering the facial aspect of the fronto-nasal process and hiding it completely so that it is not concerned in the formation of the facial surface." Herein lies the explanation of the early disappearance of the suture on the face in the human subject.

While such a condition as that described above is rare, malformations in this region of the face are relatively common. They may be ascribed to the fact that the development of this part in man differs in important particulars from that of all other animals owing to the immense increase in the size of the brain, which has resulted in shortening and bending of the cranial base and consequent retraction of the face. These changes are peculiar to man, hence as in other parts of the body where specially human attributes have called for special adaptations, there is a marked tendency for the parts concerned with the newly acquired characters to exhibit malformations.

CONCLUSIONS

1. That the lower margins of the nares with nasal spine and the bone on the face immediately below the spine are *exclusively human* and overlie the true premaxillary part, thus forming a floor to the anterior part of the nose which is absent in the apes as are also the nasal spine and narial margins.

2. That the growth of this new part which is derived from the maxillary process (Frazer) covers over the nasal portion of the premaxilla, obliterating the suture between it and the maxillary part.

EXPLANATION OF PLATE I

Fig. 1. Face view of Skull 1 showing perfectly formed narial margins and nasal spine. The crown of the right permanent canine tooth is exposed below the nasal spine and its root is visible as an elevation of the bone on the front of the maxilla.

Fig. 2. To show palate and teeth of Skull 1 with abscess cavities at roots of second bicuspid and first molar. Note absence of horizontal plates of palatine bones, and second and third molars.

Fig. 3. Profile view of Skull 1 with mandible in position.

Fig. 4. Face view of Skull 2. The union of the maxillary processes has been less complete than in Skull 1. Note absence of nasal spine and cleft in middle line between sockets for canine teeth.

Fig. 5. To show asymmetry of palate. Compare with corresponding view in Skull 1. Note presence of palatine bones and sockets for all molar teeth.

Fig. 6. Profile view of Skull 2 and mandible.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

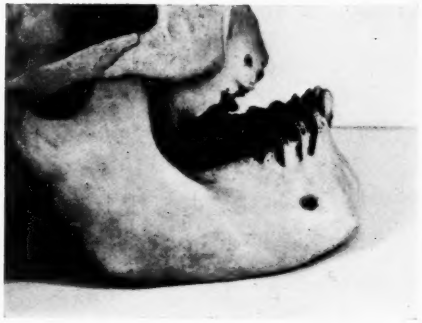

Fig. 6.

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ANOMALOUS HEART

By G. I. BOYD

This heart was obtained by the courtesy of Dr Polson, St James's Hospital, Leeds, from a child aged 3, who died of pneumonia. The viscera were transposed but the heart was only partially transposed, a condition to which reference has been made by Maude Abbott under the title "Incomplete Heterotaxy" (Osler's *Modern Medicine*, 3rd ed. 1927, vol. IV, p. 664). Left and right superior venae cavae were present and the atria formed a common chamber.

The sterno-costal surface of the heart is seen in Plate I, fig. 1. Match-stalks are placed in the right superior vena cava and in the arterial trunks arising from the aortic arch. The right ventricle has been opened up and, as is to be expected in a case of transposed viscera, gives rise to the aorta. But this "aortic ventricle" has all the appearances of a normal right ventricle, and the ascending aorta lies *in front of* the pulmonary trunk. If the words "pulmonary trunk" could be substituted for "aorta", the appearance would be that of an ordinary heart as seen from the front. The cusps in the aortic valve are placed two in front, and one behind in relation with the single anterior cusp of the pulmonary trunk. The coronary arteries arise by a common orifice from the left anterior aortic sinus. A deep pocket can be seen passing upwards, backwards and to the left under cover of the infundibulo-ventricular crest. It is an exaggeration of the small pocket normally found in this situation.

In Plate I, fig. 2, is shown the interior of the left ventricle which opens above into the pulmonary trunk. The latter passes behind the ascending aorta and is connected with the aortic arch by a patent ductus arteriosus. The left atrio-ventricular orifice has a valve of two flaps and the upper part of the cavity of the ventricle has fibrous walls like the aortic vestibule of a normal left ventricle. The trabeculae are more numerous, smaller, and more closely reticulated than in the opposite chamber. In the upper part of the ventricle, the vestibule, there is a swelling with a thin membranous wall, produced by an aneurysmal dilatation of the right ventricle at the site of the membranous part of the septum and corresponding to the pocket seen in Plate I, fig. 1.

The back of the heart is shown in Plate I, figs. 3 and 4. A rounded bundle of muscle crosses the upper part of the common atrial chamber and from it a fenestrated sheet of muscle stretches to the roof. Below the muscular bundle there is complete continuity between the two portions of the cavity. Fig. 3 shows the left half of the common atrium opened from behind, the incision having opened up the inferior vena cava entering below and the left superior vena cava entering above. A rod of plasticene lies in the groove formed by the slit-up superior vena cava. A crista terminalis will be noticed passing down the left wall of the cavity from in front of the termination of the left superior

vena cava. In front of the crest the wall shows musculi pectinati; behind the crest the wall is smooth and corresponds to the sinus venosus.

Plate I, fig. 4 shows the slit-like, valvular opening of the right superior vena cava, situated at the uppermost part of the right portion of the common atrium and surrounded by an area marked by trabeculae carneae. The pulmonary veins also open into this right portion, but the wall immediately surrounding them is smooth.

DISCUSSION

This is a case in which along with general transposition of the viscera the heart is incompletely transposed and the septum of the heart has developed irregularly. The interatrial septum is incomplete and the membranous septum between the ventricles has stretched and produced an aneurysmal swelling. In the atria the transposition is practically complete owing to the dominance of the left horn of the sinus venosus so that the left half of the common atrial chamber has all the appearances of a normal right atrium. But the right horn of the sinus venosus has persisted both in its continuity with the common cardinal vein and in its position of entrance into the primitive atrium. It has become a right superior vena cava opening into the summit of the right portion of the common atrium instead of being transformed into a coronary sinus which should have opened into the left portion. McCotter (*Anat. Record*, 1916, vol. x, p. 373) quotes cases of a similar method of termination of a persistent superior vena cava. The ventricular chambers are themselves not transposed, for each retains the characteristic features of a left and right ventricle, but functional transposition has been brought about by an anomalous development of the septum of the truncus arteriosus whereby the ascending aorta passes in front of the pulmonary trunk and the right ventricle does the work carried out by the left ventricle of a normal heart.

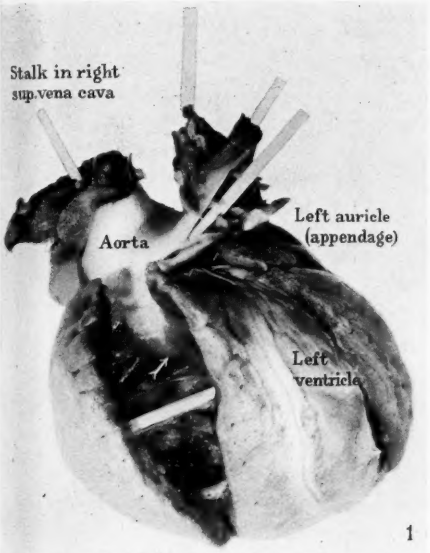
EXPLANATION OF PLATE I

Fig. 1. Front of heart. Right ventricle opened. The arrow leads to the pocket behind the supraventricular crest.

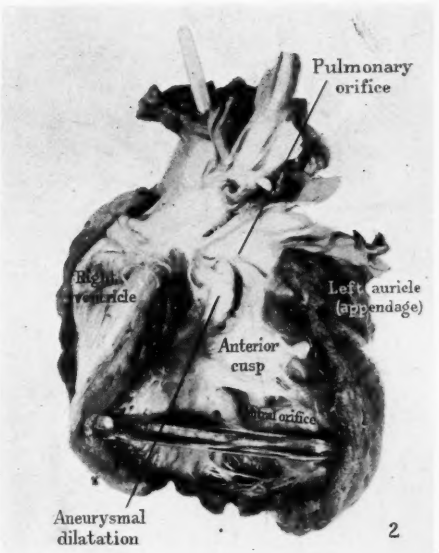
Fig. 2. Front of heart. Left ventricle opened. Shows projection of membranous septum as an aneurysmal dilatation.

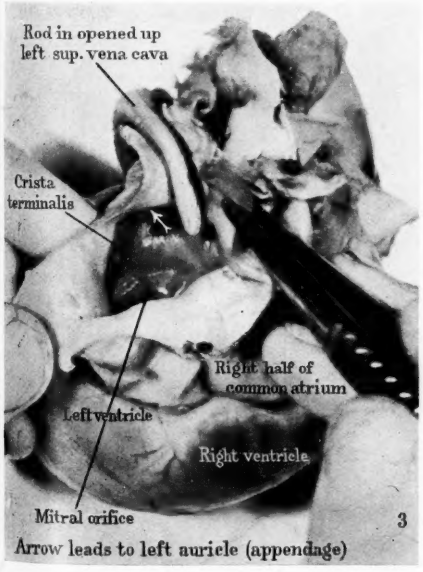
Fig. 3. Postero-inferior view of heart. Left half of the common atrium opened up. A rod is placed in the left superior vena cava. The arrow leads into the left auricular appendage.

Fig. 4. Postero-inferior view of heart. Right half of the common atrium opened up. Shows the valvular opening of the right superior vena cava.

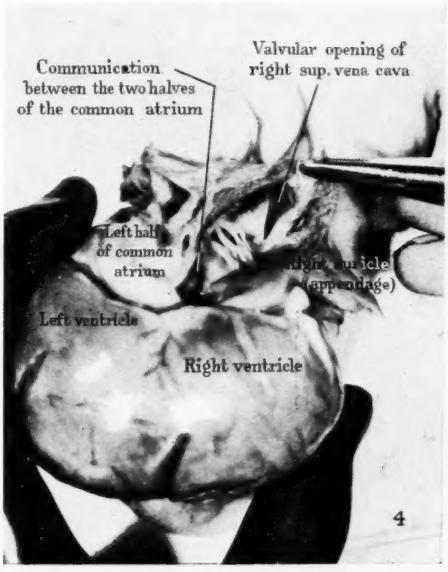


Arrow in pocket behind supra ventricular crest





Arrow leads to left auricle (appendage)


Communication between the two halves of the common atrium
Valvular opening of right sup. vena cava
Left half of common atrium
Left ventricle
Right ventricle
4

UNIV. OF MICH.

THE EXTERNAL CHARACTERS OF A SECOND AUSTRALIAN FOETUS

By FREDERIC WOOD-JONES
Melbourne

In a previous note (*J. Anat., Lond.*, vol. LXVII, pt. IV, p. 549) I described the external features of an Australian foetus of about the 17th–18th week of intrauterine life. The subject of the present note is considerably younger and, both by its general state of development and by its measurements, must be assigned to a period not later than the 9th week. The specimen was procured many years ago by the late Mr Herbert Basedow at Alice Springs and, despite its having had a rather chequered career since the death of Mr Basedow, there is no doubt about its authenticity. The condition of preservation is not perfect, the head having suffered some shrinkage and flattening, but the histological condition of the tissues is surprisingly good.

MEASUREMENTS

In the table of measurements given below I have included those of a normal white foetus of slightly larger size for comparison with the indices obtained from the Australian foetus.

Table of measurements

Measurement	Australian aboriginal	White
Rump-vertex	37	41
Suprasternale-symphysion	15	20
Arm	8	9
Forearm	6	8
Hand	5	6
Thigh	8	10
Leg	6	7
To sole of foot	−2	+2
Length sole of foot	6	7

The interest in these measurements lies in the fact that, as in the 17th–18th week foetus, the great relative length of the limbs, which characterizes the adult aboriginal, is already well marked at about the 9th week of intrauterine life.

While the upper extremity/lower extremity ratio is very nearly the same in the white and aboriginal foetuses and the arm-forearm/leg-thigh ratio is identical; the upper and lower extremity/trunk length ratios are considerably higher in the aboriginal. The hand and foot of the aboriginal are also considerably longer relative to the trunk length.

Fig. 1. General characters of Australian foetus.

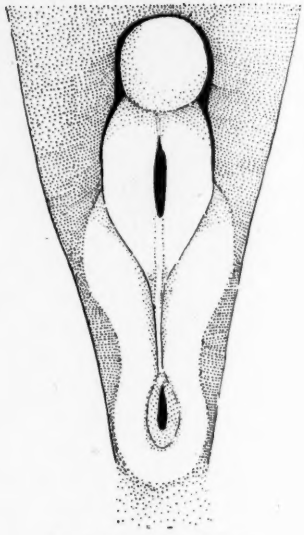


Fig. 3. External genitalia.

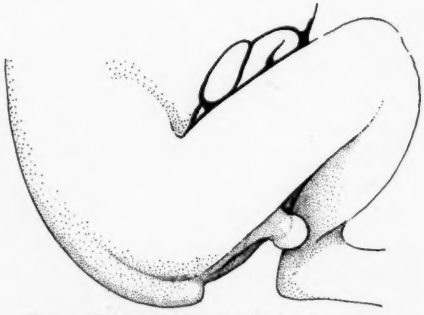


Fig. 2. Hind end of his body showing caudal extremity and external genitalia.

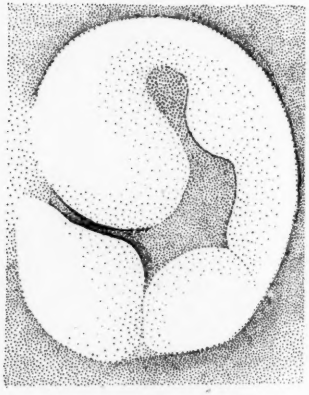


Fig. 4. Left pinna.

Table of indices

	Australian aboriginal	White
$\frac{\text{Arm and forearm}}{\text{Trunk length}}$	93·3	85
$\frac{\text{Upper extremity}}{\text{Trunk length}}$	126·6	115
$\frac{\text{Thigh and leg}}{\text{Trunk length}}$	93·3	85
$\frac{\text{Lower extremity}}{\text{Trunk length}}$	103·3	95
$\frac{\text{Arm and forearm}}{\text{Leg and thigh}}$	100	100
$\frac{\text{Upper extremity}}{\text{Lower extremity}}$	126	121
$\frac{\text{Hand}}{\text{Trunk length}}$	33·3	30
$\frac{\text{Foot}}{\text{Trunk length}}$	40	35

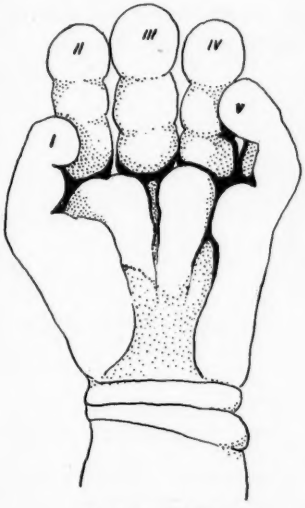


Fig. 5. Left hand.

Fig. 6. Sole of right foot.

In the general question of the antiquity of human racial differentiation it would seem to be a fact of considerable interest that the typical racial proportions of the Australian aboriginal are fully established at the 9th week, when the two proximal segments of the limbs are only 14 mm. in length.

GENERAL CHARACTERS

The foetus is 37 mm. in *R-V* length. The eyes are closed and the edges of the lids are smoothly covered by a growth of epitrichium. The mouth is open: the anterior nares are occupied by epithelial plugs. The vitelline loop is exposed by the tearing of the cord from the body (see Fig. 1). The caudal process is unusually well marked and, although there is only a very short free tail, the prominence of the caudal tubercle is well developed (see Fig. 2). The development of the external genitalia appears to be somewhat precocious. The sex is obviously male. The ostium urogenitale is situated about midway on the body of the penis and persists only as a narrow cleft in front of the closed inner genital folds (see Figs. 2 and 3). The whole genital tubercle appears large when comparison is made with white foetuses of about the same size.

The pinna, on the other hand, would appear to be somewhat rudimentary in development for a 37 mm. foetus with closed eyes. The tuberculum tragicum is well defined and the hyoid derivatives have taken definite shape; but the pinna itself is but little sculptured from the general skin field (see Fig. 4).

On neither fingers or toes is there any sign of developing nails, even in microscopic sections. Upon the palm, the 4th, 3rd and fused 1st and 2nd pads are distinct and on the sole all four pads are separate and well defined. The central digit of both hand and foot is the longest (see Figs. 5 and 6).

ANATOMICAL NOTES

A PECULIAR RELATION OF THE FEMORAL HEAD OF THE BICEPS FEMORIS TO THE COMMON PERONEAL NERVE

By Y. H. AASAR

This case was met with in the course of the dissection of the left lower limb. The sciatic nerve has divided high up at its exit from the greater sciatic foramen. The common peroneal nerve describes a curious twist round an additional tendinous head of the biceps femoris which arises from the base of the greater trochanter. The nerve is definitely impressed by the tendinous slip. Figure 1, originally made from the specimen by Dr S. Farag and then redrawn by Mr N. Strekalovsky, shows the relation very clearly. This arrangement might have given rise to symptoms during life, the cause of which could never have been discovered.

This peculiar case can be explained by the migration of the femoral head of the biceps which was originally a gluteal muscle arising from the caudal vertebrae and

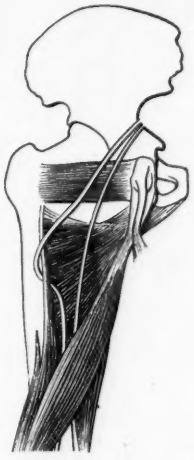


Fig. 1. Drawing of back of upper part of lower limb to show the peculiar relation of the femoral head of biceps, arising from the base of the greater trochanter, and the common peroneal nerve.

inserted into the crural fascia. It is suggested that the actual migration has taken place in this very specimen during development. The tendinous slip as it passed laterally came to lie deep to the common peroneal nerve but, immediately below, it remained superficial to the nerve.

On the right side there is a similar tendinous slip which reaches the gluteal ridge on the back of the femur. There is no twist in the nerve which lies medial to the slip. On the same side the pyriformis and the superior gemellus are not normal; the former blends in part with the gluteus minimus while the latter is absent and seems to be represented by a slip that arises from the upper border of the greater sciatic notch, passes through the nerves arising from that notch and passes down to be lost in the vicinity of the lesser notch. It is supplied by a branch from the nerve to the obturator internus muscle.

My thanks are due to Prof. D. E. Derry for the interest he took in the case.

ABNORMALITIES OF THE LUMBRICAL MUSCLES OF THE HAND

By K. F. RUSSELL and SYDNEY SUNDERLAND
Department of Anatomy, University of Melbourne

The following abnormalities of the lumbrical muscles were observed in the superior extremities of an adult male dissecting room subject.

In the right hand the first and second lumbrical muscles were entirely absent. The third and fourth were present but showed the following variations: the third lumbrical arose in the middle of the forearm as a thin narrow tendon from the volar

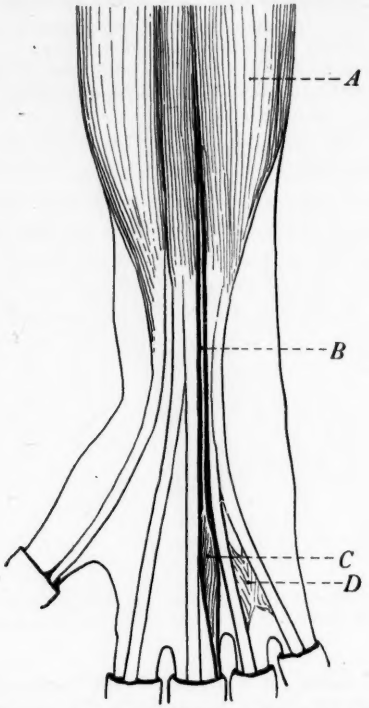


Fig. 1. Authors' case. *A* = flexor profundus digitorum; *B* = tendon of abnormal third lumbrical; *C* = muscular belly of same; *D* = abortive formation of a normal fourth lumbrical. An approximately similar conformation of the second and third lumbricals has been observed by Dambrin(1).

surface of that part of the main muscular mass of the flexor profundus digitorum muscle destined for the medial three fingers. It passed distally, superficial to the flexor profundus mass, and crossed the wrist into the palm medial to the profundus tendon for the middle finger. Here the tendon gave rise to a small discrete fleshy belly which, at the level of the web-space between the middle and ring fingers, became tendinous and passed to be inserted into the tendon of the third dorsal interosseous muscle just distal to the base of the proximal phalanx of the middle finger (Fig. 1).

Thus both the origin and insertion of this third lumbrical are atypical. In the fourth interspace a thin muscular sheet was observed arising from the adjacent margins of the tendons of the deep flexor to the ring and little fingers and stretching across the triangular interval formed by these tendons at their divergence to their respective fingers. This muscle mass, which gave rise to no tendon, is to be looked upon as an abortive attempt at the formation of a normal fourth lumbrical, the origin of which alone is to be regarded as normal (Fig. 1).

In the left hand the first and second lumbricals were absent as on the right side. The third lumbrical was again represented as in the right arm with this difference, that the tendinous slip arising from the volar aspect of the composite mass of the flexor profundus digitorum took origin opposite the pisiform bone, before the profundus tendons to the ring and little fingers were differentiated. Unlike the anomalous slip on the right side, no muscular belly developed, and the tendon was inserted into the superficial surface of the transverse metacarpal ligament just medial to the base of the proximal phalanx of the middle finger. The fourth lumbrical was similar to that on the right side, but not so well marked.

Literature

A. Macalister(2), in a preliminary paper on muscular abnormalities in 1866, states that the palmar lumbricals vary frequently in their origin, principally by the occurrence of additional heads of origin from neighbouring tendons. He records that the third lumbrical is by far the most variable in the proportion of 2:1 as compared with others. In his paper on muscular abnormalities(3) published in 1875 he has collected and recorded all the variations of these muscles previously described.

Discussion

From a study of the literature (the papers consulted are listed at the end), the variations in the lumbrical muscles may be divided into the following well-defined groups:

(1) Those cases in which one or more of the lumbricals presents an abnormal or accessory head of origin. Such have been recorded coming from the flexor sublimis digitorum, flexor profundus digitorum and flexor longus pollicis in the forearm as well as from the sublimis and profundus tendons in the hand and from the interossei and lumbrical muscles.

(2) Those cases showing abnormal insertions, single or multiple, usually taking the form of additional tendinous slips passing to adjacent fingers.

(3) Cases showing an abnormal origin associated with an atypical insertion. These must be singularly rare, the only recorded cases being that described at the beginning of this paper and that described by Wood(4) (quoted by Macalister).

(4) Those cases showing numerical variation of muscles.

Summary

1. An example of unusual variation of the lumbrical muscles of the hands is described.

2. The literature dealing with variations of these muscles is reviewed.

We wish to record our thanks to Prof. Wood-Jones for the diagram which accompanies this paper.

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(1) Dambrin, P. (1930). *Ann. Anat. Path. méd.-chir.* vol. VII.
(2) Macalister, A. (1866). *Proc. R. Irish Acad.* vol. IX.
(3) —— (1875). *Trans. R. Irish Acad.* vol. XXV.
(4) Wood, J. (1866). *Proc. roy. Soc.* vol. XV.

NOTE ON COUNTERSTAINING WEIGERT-PAL SECTIONS OF THE CENTRAL NERVOUS SYSTEM

By F. DAVIES
Anatomy Department, University of Sheffield

The agents commonly employed for counterstaining histological sections of the central nervous system after treatment by the Weigert-Pal (or Kultschitsky-Pal) technique, with a view to demonstrating the nerve cells and their processes, are neutral red, various carmine preparations (e.g. alum carmine, picrocarmine, aceto-carmine, etc.), or safranin.

Of these, alum carmine, prepared as recommended by Anderson (1929), gives very good results, the nerve cells being crisply and deeply stained. But unfortunately the acetic acid in this preparation tends to decolorize the haematoxylin-stained myelin sheaths of the nerve fibres, particularly if the counterstaining process be prolonged. Even using the times recommended by Anderson, namely 2–3 hours at 50° C., a distinct paling of the medullated sheaths occurs. To obviate this, and to retain the crisp and brilliant staining of the nerve cells, the following method of counterstaining is recommended.

After washing in tap water, subsequent to differentiation in the Weigert-Pal technique, the sections are immersed in alum carmine (Anderson's formula) for 2 hours at 37° C. They are then washed in several changes of distilled water and transferred to neutral red solution for 1–2 min. (neutral red 0·5 g., acetic acid 1 per cent 5·0 c.c., distilled water 100 c.c.). They are finally washed in tap water to remove excess of stain, dehydrated through 70, 90 and 96 per cent alcohols, cleared in carbol-xylol and then in xylol, and mounted in balsam. (Prolonged clearing in carbol-xylol tends to remove the neutral red stain and prolonged immersion in xylol tends to crease the celloidin section.)

By this method, the nerve cells and their nuclei are crisply and deeply stained, the tigroid bodies clearly exhibited, and no appreciable paling of the myelin sheaths occurs. The neutral red deepens the staining of the nerve cells by the alum carmine and lessens considerably the time necessary for immersion in the carmine solution. It is claimed that this method gives an improved final total result over the other techniques mentioned.

I wish to thank my technical assistant, Mr J. T. Hall, for the experimentation which resulted in the evolution of this technique.

REFERENCE

Anderson, J. (1929). *How to Stain the Nervous System.* Edinburgh: Livingstone.

THE QUADRATE TUBERCLE OF THE FEMUR

By SYDNEY SUNDERLAND
Department of Anatomy, University of Melbourne

Reference to descriptions in current textbooks of anatomy concerning the relationship of the quadratus femoris muscle to the quadrate tubercle reveals a condition which differs from that usually found by dissection, whilst the gross anatomy of the tubercle itself receives very little attention. Thus in Gray(3) it is stated that "a little above its middle (trochanteric crest) it presents a low rounded elevation, sometimes termed the quadrate tubercle. The tubercle itself, and a portion of the bone below, receive the insertion of the quadratus femoris muscle". Morris(5) makes the statement that "above the middle of this line (intertrochanteric crest) is an elevation termed the tubercle of the quadratus for the attachment of the upper part of the quadratus femoris" whilst on p. 464 the quadratus femoris is said to insert "into the vertical ridge which terminates above on the inferior dorsal angle of the great trochanter". Cunningham(1) says: "The low, rounded swelling on or alongside the crest, on the back of the trochanter major is called the quadrate tubercle because part of the quadratus femoris muscle is inserted into it." The muscle is also said to be inserted into the quadrate line of the femur below the tubercle. Poirier & Charpy(6) illustrate the quadrate tubercle but do not describe it. Of the quadratus femoris they say, "De là, les fibres se dirigent transversalement en dehors pour aller s'insérer non à la ligne intertrochantérienne postérieure, mais à une ligne rugueuse qui, continuant l'interstice de la ligne âpre du femur, aboutit au tubercule de l'angle postéro-inférieur du grand trochanter." "Quelquefois, le muscle se dédouble près de son insertion fémorale: ses fibres postérieures, les plus nombreuses, vont à l'insertion que j'indique, tandis que les antérieures s'arrêtent à la ligne intertrochantérienne." Frazer(2) associates the tubercle and the muscle in the following manner: "The posterior intertrochanteric line is part of the trochanteric system and marks the pull of the quadratus." "The quadrate tubercle is that part of the trochanteric mass on which the pull of the muscle is directly exerted, and it is thus of the nature of a primary marking." Macalister(4) has described the condition thus: "Between the trochanters posteriorly is a rounded intertrochanteric line, at the middle of which is a small roughness from which a faint, short line descends vertically to the level of the lesser trochanter. This is the linea quadrati, which receives the insertion of the quadratus femoris."

Routine dissection of the region concerned showed that none of the above descriptions was a true representation of the commonest condition found. On account of these discrepancies, the anatomical features of this bony marking were investigated in a series of 100 adult femora whilst the relationship of the muscle to the tubercle was ascertained from a study of the soft parts in 10 foetal and 20 adult dissections. The investigation was carried out in three parts. In the first part the situation and the characteristics of the tubercle in the adult bone were determined; in the second the relationship of the epiphyseal line of the greater trochanter to the tubercle was ascertained, and in the third the relation of the tubercle to the quadratus femoris was determined by dissection. The adult bones examined were those of Australian aboriginals as were four of those possessing epiphyseal lines; the dissections, however, were made on white material. This unfortunately, though definitely, prevents any direct comparison and correlation between the various parts of the investigation.

The tubercle

The tubercle was present on the intertrochanteric crest in every femur of the above series and was so well developed that no doubt was left as to its actual presence. The summit of the tubercle, however, was not *on* the crest but just lateral to it. In an attempt to determine the site of the tubercle more accurately the following measurements were taken. The distance between the point where the crest joined the upper border of the trochanter and the summit of the lesser trochanter was divided by the distance between the summits of the lesser trochanter and quadrate tubercle and the ratio so obtained compared in the various femora of the series. Such measurements were obviously only approximate, yet the ratio obtained in all

Fig. 1. Diagram illustrating the insertion of the quadratus femoris muscle as revealed by dissection. The main insertion is indicated in black, the superficial off-shoot in horizontal and the deep offshoot in vertical lines. (Drawing by Prof. Wood-Jones.)

cases was relatively constant. In sixteen specimens the ratio was 1·4, in thirty-two, 1·5, in thirty-five, 1·6, in nine, 1·7, and in four, 1·8, thus giving a mean of 1·5. In four specimens destruction of the lesser trochanter prevented the necessary measurements from being made. From these measurements it is certain that the tubercle is always situated above the mid-point of the intertrochanteric crest and that it is to be found at approximately the junction of the upper one-third with the lower two-thirds of the crest. The tubercle varies in size and is not always localized to the crest. In the majority of cases the elevation involved the adjacent area of the posterior surface of the greater trochanter and in a few cases it actually passed over on to the adjacent portion of the neck of the bone. It is to be noted that these extensions medially and laterally always take place along the site of the epiphyseal line.

Relationship to the epiphyseal line of the great trochanter

In the only three sets of bones available for study showing this epiphyseal line, the line passed directly through the tubercle, which thus forms by far its best landmark on the posterior surface of the adult bone.

RELATIONSHIP TO THE QUADRATUS FEMORIS MUSCLE

The tubercle was present in the foetus though, compared with its condition in the adult, it was ill defined. In all the cases dissected it was situated close to, if not at, the mid-point of the intertrochanteric crest. The main mass of the muscle was found to be inserted, without exception, along the intertrochanteric crest. The upper fibres ceased a short distance before the point where the crest joined the superior border of the trochanter but the lower fibres passed down along the posterior margin of the upper half of the lesser trochanter, where they blended inferiorly with the upper fibres of insertion of the adductor magnus. The insertion also extended medially into the fossa overlapped by the crest whilst some of the fibres were traced as far up as the posterior surface of the digital fossa where they were closely associated with the fibres of the obturator externus muscle. In one case the insertion of the main mass was so far medial to the crest that it was liable to confusion with the obturator externus. Laterally, fibres passed over on to the tubercle and as far down as the lesser trochanter; these lateral fibres had the weakest attachment and could be separated quite easily from the bone: the remainder of the muscle was strongly attached by muscle fibre. In the adult the tubercle was again present in all members of the series and could be detected without difficulty. The ratio taken in five specimens gave a value of 1·5 in one and 1·4 in the remainder. The insertion of the muscle was relatively constant and took the following form: The main mass of the muscle inserted by a fine admixture of muscle and tendon along the intertrochanteric crest, but inferiorly it passed off the crest on to the shaft of the bone as far down as the level of the lesser trochanter. Superiorly the muscle never reached the postero-superior angle of the trochanter, but fibres left the deeper surface of its upper portion to insert into the inner lip of the crest and to come into close contact with the obturator externus tendon as it passed upwards and outwards to insert into the digital fossa. Inferiorly fibres from the deep surface inserted as far medially as the lateral margin of the lesser trochanter. At the level of the tubercle tendinous fibres from the superficial surface of the main mass passed out constantly to insert by a thin tendinous sheet into the inner aspect (rarely the summit) of the tubercle. In only three cases did the main muscle mass pass so far laterally as to involve the summit of the tubercle, and even in these cases deeper fibres were inserted along the crest as far down as the lesser trochanter. In no case in this series was the main mass inserted lateral to the tubercle although, in a few, dissection revealed isolated tendinous fibres passing out over it to be inserted into the posterior surface of the trochanter. Thus in this series the double muscle described by Poirier & Charpy is found to be the one which is normally present, though here the deeper portion, which is their accessory insertion, forms the main part of the muscle. In no case did the tubercle form the upper limit of the insertion of the muscle which, on the contrary, was constantly found above it. The condition found certainly suggests that the muscle is responsible for the formation of the crest as stated by Frazer, though his statement that "the tubercle is that part of the trochanteric mass on which the pull of the muscle is directly exerted" received no confirmation from this investigation, in which the tubercle was associated, not with the main portion of the muscle, but with a lateral offshoot from it. However, it is extremely likely that changes peculiar to, and taking place at, the epiphyseal line are also responsible for some of the prominence of the tubercle. This receives confirmation from the fact that, in the series demonstrating epiphyseal lines, limited though it was, the tubercle was intimately associated with that line. Moreover in those cases in which extensions of the tubercle from the crest were present they were always found along the path of the growth line, and again, the tubercle is often larger than can be accounted for by the site and nature of the muscle insertion. There seems little doubt, however, that the muscle is responsible for the early appearance of this bony elevation.

SUMMARY

1. An investigation into the nature of the quadrate tubercle is described in which that bony marking is found to be constantly present, and to occur at a relatively fixed point along the intertrochanteric crest.

2. The tubercle is found to be a reliable landmark to the epiphyseal line of the great trochanter.

3. The relationship of the quadratus femoris muscle to the tubercle and the intertrochanteric crest is described, whence it is concluded that the tubercle is a muscular marking due, not to the main muscular mass, which is responsible for the crest, but to a smaller offshoot from it.

REFERENCE

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REVIEW

Synthetische Morphologie der Niere des Menschen. Bau und Entwickelung dargestellt auf neuer Grundlage. Von Prof. Dr MARTIN HEIDENHAIN, Tübingen. (Leiden: E. J. Brill.) 1937. Pp. xvi+270, 90 figs. Price 10 guilders; in Leinwand 12 guilders.

This is both an interesting and important book because by observation and logic it does something to liberate us from the restrictions of the cell doctrine. It has always been difficult to pass from the division of separate cells to the functional and morphological units of the body. Integration and unification are profound problems for the understanding of the metazoic body.

The author uses the kidney as his type organ and begins with the single dividing cell as the unit. Chapter by chapter he presents his results, proceeding from earlier to later stages, from the single cellular to the multicellular unit, until the formation of the kidney is complete.

At every stage he shows how the unit of division originally a cell becomes a larger and larger group. Each grouping presents a characteristic form and shape demanding a nomenclature that fits into the sequence of events in the formation of the kidney. Thus he presents us with a synthetic morphology.

Though in this monograph the author's exposition is confined to the kidney, his other writings deal with similar principles in the formation of the organs.

The work and the conceptions elucidated in this monograph seem to us of great importance in scientific morphology.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

November 1937

RECORDED BY
A. J. E. CAVE

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

NOVEMBER 1937

The Annual Meeting of the Society was held in the Department of Anatomy, London School of Medicine for Women, at 3 p.m., on Friday, 19 November 1937, the President, Professor ALEXANDER LOW, in the Chair.

Professor M. F. LUCAS KEENE and Dr E. E. HEWER read a paper on "The connexions of the posterior commissure", which will be submitted for publication in the *Journal*.

Professor LE GROS CLARK commented upon the archaic nature of the posterior commissure. He inquired whether this commissure (more correctly, "decussation") was structurally distinct from that of the posterior colliculus: he asked whether any neurocytological evidence existed indicating connexions between the posterior commissure and both the subcommissural organ and the pineal. The presence of certain optic tract fibres, which had been previously described as crossing in the posterior commissure to gain the contralateral oculomotor nucleus, had not been confirmed by experimental inquiry.

Professor DURWARD asked whether the interstitial nucleus manifested any midline crossing. Dr UNA FIELDING inquired whether any connexion had been observed between the cranial extremity of the posterior longitudinal bundle and the hypothalmus. Professor LUCAS KEENE, in reply, stated that the posterior commissural fibres blend with those of the tectal commissure, that in man, the subcommissural organ gradually vanishes as development proceeds, that a small but definite myelinated fibre-connexion with the pineal is to be encountered, but that no information could be offered from the present study touching the interstitial nucleus or the posterior longitudinal bundle.

Professor A. B. APPLETON read a paper on "The level of the xiphisternal joint and diaphragm in the living subject."

The relative levels of the xiphisternal joint, right dome of the diaphragm and the ninth thoracic spinous process have been examined radioscopically in a series of fifty-two young students of both sexes in the erect posture. Pieces of lead were applied to mark the position of bony points. Only the central beam of X-rays were employed, in order to minimize distortion.

The xiphisternal joint was in most subjects situated considerably lower than the generally accepted level in the cadaver. This difference is probably mainly the expression of an age difference. In the female the xiphisternal joint was on the average about 5 cm. below the ninth thoracic spinous process, in the male about 2 cm. below that process. These levels correspond approximately with the disk below the 11th vertebra in the female, and the disk below the 10th vertebra in the male.

In a movement of deep inspiration the xiphisternal joint moved upward till in the average female it was approximately opposite the ninth thoracic

spinous process; in the male it reached a level 1 cm. higher. The displacement is brought about by costal movement, but in some subjects extension of the vertebral column contributes to the displacement. The angular movement of the vertebral column may attain an angle estimated at 10°.

The right dome of the diaphragm was situated at a higher level in the female than in the male. In both sexes it was lower than in the cadaver. The alteration in level of the diaphragm during a movement of inspiration is the algebraic sum of two quantities: (1) the distance through which the diaphragm is lowered relative to the chest wall, and (2) the distance through which the chest wall is raised. In these subjects the average alteration in level of the diaphragm was a lowering during inspiration by about 0·5 cm.

Graphs show the movements of the xiphisternal joint and diaphragm during inspiration in a number of subjects. Individual differences are discussed with reference to the different types of body build and to costal and diaphragmatic types of respiration.

Mr WHILLIS asked whether note had been taken of the antero-posterior excursion of the xiphisternum: he had noted extreme individual variation in sternal movements, and had now abandoned radiographic examination of such movements in favour of mechanical methods. Professor LOCKHART stressed the importance of the transverse thoracic diameter in any estimation of costo-sternal and diaphragmatic movements: costal breathing was quite commonly encountered in modern athletic women.

In reply Professor APPLETON said that antero-posterior measurements had been taken to determine the extent of spinal movement during respiration. Narrow-chested subjects showed poor movement of both diaphragm and costal margin, whilst broad-chested subjects showed good movement of both: corresponding amplitudes of costal and diaphragmatic movement.

Professor F. DAVIES demonstrated a large steel microtome knife and gave details of its advantages and manufacture.

Dr A. J. E. CAVE demonstrated the "Skeletal development in a eunuch".

Gonadal influence upon ossificatory pattern is exemplified by the skeleton of an aged Egyptian eunuch (No. 4846.1, Path. Ser., R.C.S. Mus.). The sagittal, coronal and lambdoid sutures are unobliterated; the edentulous alveolar processes are fully preserved; the sternum is 3-pieced and the sacrum incompletely consolidated; the pelvis is characteristically male in type. Epiphyseal union is remarkably retarded in both the axial and appendicular skeleton. Some epiphyses have never united; others are still in process of uniting; others again reveal distinct evidence of their former independence.

The first group includes epiphyses of the scapular vertebral border, the sternal end of clavicle, and the rib heads.

The second group includes epiphyses of iliac crest, conjoint rami, acromial end of clavicle, spinous and transverse processes, tibial and fibular heads.

The third group embraces the proximal humeral, distal radial and ulnar, and medial epicondylar epiphyses; those for head, great trochanter and distal end of femur, and the distal tibial and fibular epiphyses.

Delay in epiphyseal coalescence is correlated with an obvious increase of stature, and a marked lengthening of the long bones of the limbs.

Professor HARRIS remarked that suppression of epiphyseal union had been recognized as non-hormonal in the experimental white rat. Professor KIRK

commented upon the growth increments at the epiphyseal ends of the long bones as distinct from the non-union of epiphyses. Dr HAINES described two mechanisms for growth suppression, one peculiar to fishes, the second peculiar to certain reptiles, mammals, and birds.

Dr A. J. E. CAVE read a paper on "The vertebra critica".

Atlas and axis are vertebrae profoundly modified for their role as handmaids to the cranium, wherewith they form a physiological entity, the "cervico-cranium". The first typical vertebra is C.V. III, occupying the critical site of union of the mobile "cervico-cranium" with the more stable column proper. The critical nature of C.V. III is reflected in the anatomy of both the normal and the irregularly segmented backbone. In both it manifests a predilection for coalescence with the axis. Such fusion (which may be pre- or post-natal, partial or entire) represents an attempt at the formation of a "cervical sacrum", to guarantee cervico-cranial stability and to secure the uninterrupted transmission caudalwards of cranial weight.

The normal development and anatomy of the third cervical vertebra was reviewed and specimens and illustrations of its fusion with the axis were exhibited, including thirty-eight such cases from the literature and twenty-four cases collected or examined personally. The occurrence of a similar vertebral concresence was reported in various lower animals (orang, dog, manatee, porcupine, armadillo and sloth).

Professor R. D. LOCKHART demonstrated a method of "Mobile articulation of the shoulder girdle and other joints".

A method used in the articulation of a skeleton in the Anatomy Department, Aberdeen, was described, through the courtesy of Professor LOW who readily permitted a complete examination of the specimen. This method, which involves the use of concealed springs and wired catgut "G" strings, has the advantages of durability and accuracy.

The method, as used in the long bones, is illustrated in Figs. 1, 2 and 3. The joints are mobilized by compression springs in the bones, and the teaching value of the method in the foot is to be demonstrated with regard to the action of the calf muscles and the effects of high heels.

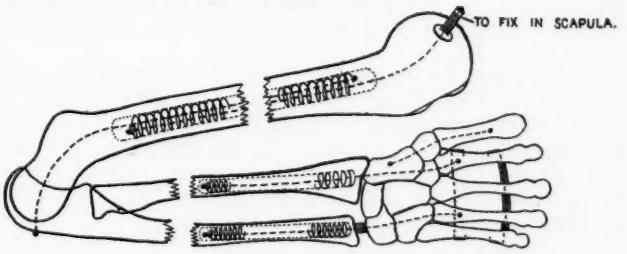

Fig. 1.

The shoulder girdle may be quickly mobilized by threading a string through the articular surfaces of the clavicle and manubrium, then through a compression spring concealed behind the sternum. An additional refinement is to thread the acromion to the clavicle through a compression spring in the clavicle,

or this spring, threaded by two strings, may operate both clavicular joints (Fig. 2). While all movements may now be executed, most probably the suggestions in the next paragraph make an additional appeal to the teacher, but, if not, then a very small piece of plastic wood fixed only to the costal end of the first rib, by way of ligament, unobtrusively prevents the weight of the arm swinging the clavicle forwards, in the stationary skeleton. Two extensile

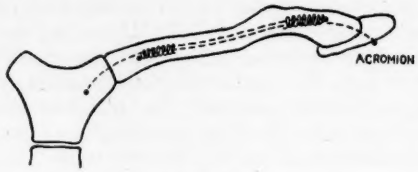

Fig. 2.

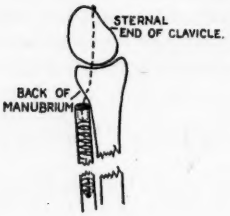

Fig. 3.

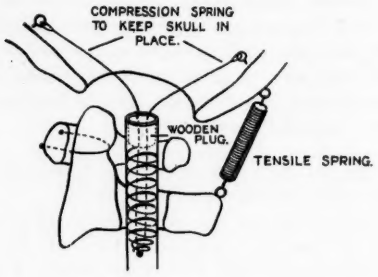

Fig. 4.

springs indicating rhomboid and serratus anterior lines of pull closely apply the scapula to the chest, and give a graphic demonstration, by their extension and contraction, of the synchronous action of these muscles in elevation of the upper limb. The upper and lower fibres of trapezius may be similarly indicated. This affords the student, and possibly the teacher, an easier, quicker and more vivid appreciation of the action of the muscles than may otherwise be obtained.

Dr D. Stewart and Dr W. Lewinsky read a paper on "The innervation of the gum", which will be submitted for publication in the *Journal*.

Mr Richardson intimated a modification of staining procedure likely to prove valuable in determining whether or not the gingival nerve-endings were encapsuled. Professor Woollard surveyed current views and knowledge of the nature of nerve-endings, and discussed the great difficulties of interpretation with which the problem of sensibility was beset.

Professor W. E. Le Gros Clark and Dr L. H. Dudley-Buxton read a paper on "Studies on the growth of the finger-nails".

A series of observations have been made on the rate of growth of finger-nails in normal undergraduates and school children, with a view to providing a standard of comparison for nutritional studies. These observations have shown that the ratio of the rate of growth in the different digits is constant, and that

the rate of growth is greatest in the third digit and least in the fifth digit. The thumb nails grow slightly less rapidly than the second and fourth digits. Contrary to the usual statement, there is no significant difference between right and left hands. There is a slight sexual difference in children, growth being more rapid in boys. No age difference was observed within the limits of the age groups studied, but there is a pronounced seasonal variation. Nail-biting is associated with a remarkable increase in the rate of growth of the nails, which is on the average 15–20 % greater than in normal cases. The individual variation in the growth of the finger-nails in normal subjects is approximately 15 %.

Dr GRAY inquired as to the nature and significance of the white areas often encountered in the pink part of the nails: if these represented pathological changes, their presence vitiated the use of the lunular edge as a fixed limit in nail mensuration. Professor BLAIR inquired whether different modes of nail trimming affected the rate of nail growth. Dr O'CONNOR remarked upon the association of nail growth and deficiency of gastric secretion. Professor WOOLLARD contrasted ectodermal growth and ossification as biological processes: vitamin A seemed essential to keratin formation. Removal of pineal gland had been alleged to affect growth of hair but his own experiments had not supported this.

In reply, the authors stated the significance of nail "spots" was unknown: the edge of the lunule, however, corresponded to the edge of the nail matrix and was certainly a fixed limit. Mode of manicure did not effect rate of nail growth. Though retardation of nail growth had been attributed to nerve-injury it had been shown (by Head and Sherren) that the real cause of such retardation was the immobility of the limb during treatment, and not the actual neural trauma.

Dr R. W. HAINES read a paper on "The structure of the epiphyses in *Echidna*".

In a femur of a young *Echidna* the cartilaginous epiphyses of the head and condyles contain vascular canal systems which have the typical mammalian structure, but which spring from the marrow tissues of the shaft and not from the perichondrium. These probably originate in the usual way from the perichondral tissues, and later acquire a secondary continuity with the shaft through the growth cartilage, as to the perforating canals of other mammals. The secondary centres occur as large areas of hypertrophied cartilage, later eroded and ossified at several separate parts partly directly by the perichondrium, and partly by the tissues of the cartilage canals. The calcified areas are reptilian in arrangement, the presence of canals is probably a primitive mammalian feature, while their attachment to the shaft is a special peculiarity. The mode of ossification in *Echidna* suggests that the phylogenetic change from a perichondral mechanism to that involving cartilage canals occurred gradually while the calcified areas were still large.

Professor HARRIS suggested the investigation of the young human costochondral junction, as a field yielding a variety of histological patterns, including possibly the same dual ossificatory mechanism as described in this communication.

Professor H. A. HARRIS read a paper upon "The sternum".

Radiographic evidence is produced to show that the number of segments in both the presternum (manubrium) and mesosternum (gladiolus) undergoes

reduction in many mammalian forms: this reduction may occur in the osseous, or in the cartilaginous, stage.

The reduction in total length of the mammalian sternum proceeds cranially in association with an increased mobility of the head and neck; caudally in association with the raising of the diaphragm, related possibly to a large full-term pregnant uterus. In the sheep the fifth segment of the mesosternum is removed either by absorption or by fusion with the fourth segment, so that ribs 5, 6 and 7 articulate with the adult fourth segment. This process is seen in carnivores as well as ruminants. It occurs also in certain Old World and New World monkeys.

In the gibbon (*Hylobates*), in which the sternal angle and manubrio-gladiolar joint are at the level of the third costal cartilage, the functional manubrium is formed by the fusion of the osseous centres of the first segment of the true mesosternum with those of the presternum proper. This condition is normal in the gibbon, in which the number of ribs is thirteen. The condition is also seen in man, sometimes in association with thirteen ribs, and may be regarded as a gibbon-like character.

In one gibbon, wherein fourteen ribs are present, the functional manubrio-gladiolar joint is at the level of the fourth costal cartilage, so that the manubrium results from the fusion of the presternum with the first and second mesosternal elements. Another gibbon, having twelve ribs, presents the typical human type of sternal angle at the level of the second costal cartilage and of the fourth segment of the mesosternum articulating with the fifth, sixth and seventh costal cartilages.

Arbuthnot Lane described a gibbon-like sternum in man associated with the presence of a third head of the biceps and a chondro-epitrochlearis muscle—two muscles normally present in the gibbon. The anthropoid sternum often presents a further degree of reduction caudally in that the mesosternum consists of three elements, of which the third and last articulates with the fourth, fifth and sixth ribs, the seventh rib not reaching the sternum: this condition is rarely seen in man.

It is probable that the process of reduction of the fifth segment of the mesosternum in man (or of the fifth and fourth segments in certain anthropoids) occurs in the pre-osseous or cartilaginous stage. There is some evidence, based on certain abnormal sterna, that the process of reduction of mammalian mesosternal elements may show a shift cranially. This is probably dependent to some extent on both the actual dimension of the mesosternal segments and the calibre of the anterior intercostal branches of the internal mammary artery. This process of absorption in cartilage is at present being studied in the chondrocranium, mandible, branchial arches and in the limbs of certain mammalian embryos.

Dr Strauss commented upon the association of ribs and sternal segments in the *Lorisidae* and upon the musculature of the gibbon, in which animal a tricipital M. biceps brachii was purely anomalous.